Anterior Cingulate Cortex and Benefit of Predictive Cueing on Response Inhibition in Stimulant Dependent Individuals

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**Background:** Methamphetamine dependence (MD) is associated with impaired response inhibition and with structural abnormalities and functional hypoactivity in the anterior cingulate cortex (ACC). The need to inhibit behavior is often forewarned by cues that do not call for immediate inhibition. We sought to determine whether such cues would engage the ACC and improve inhibition in MD individuals.

**Methods:** We used functional MRI to measure ACC activation during performance of a go/nogo response inhibition task in which certain go stimuli (cues) were more likely than others (noncues) to be followed by nogo trials. Nineteen MD individuals (inpatient treatment, 25–50 days abstinence) were compared with 19 age- and education-matched healthy comparison (HC) subjects.

**Results:** MD and HC groups had statistically comparable performance, but only MD participants showed an ACC response and lower false alarm rates associated with cues as compared with noncues. Cue-related ACC activity in MD subjects was positively correlated with this cue-related improvement in inhibitory performance.

**Conclusions:** The ACC, an area associated with error detection and response conflict, may predict the degree to which advance warning may attenuate MD individuals’ difficulty with response inhibition.

**Key Words:** Anterior cingulate cortex, drug dependence, fMRI, go/nogo, methamphetamine, response inhibition

Methamphetamine dependence is an important public health problem associated with impaired cognition (1), including failures of inhibition, i.e. the ability to withhold a thought, feeling or action. DSM-IV drug dependence (2) points to inhibitory failure with criteria that focus on compulsive drug use despite both knowledge of adverse consequences (e.g. health, legal, and social problems) and a desire to quit. The ability to inhibit responses is commonly assessed in the laboratory using go/nogo tasks or the stop-signal task (3). In these tasks, go stimuli are frequent and signal the need to respond while stop signals and nogo are comparatively infrequent and instruct the subject not to respond. This produces conflict because the high frequency of go stimuli leads to a prepotent tendency to respond, which must be inhibited on exposure to the uncommon stop or nogo stimuli. Recently, abstinent methamphetamine-dependent subjects have demonstrated worse performance than comparison subjects on a stop-signal task (4), while other studies have found inhibitory difficulties in chronic cocaine users with stop-signal (5) and go/nogo paradigms (6,7).

Previous investigations with active cocaine users (i.e., who had used within the past few days, on average) have shown not only impaired inhibitory performance but also anterior cingulate cortex (ACC) hypoactivation during performance of a go/nogo task (6,7). Meanwhile, a number of studies also have demonstrated ACC abnormalities in individuals with methamphetamine abuse or dependence, consistent with methamphetamine’s neurotoxic effect on dopaminergic neurons, which in humans enervate the ACC more densely than any other cortical structure (8). For instance, subjects with methamphetamine dependence show decreased levels of N-acetyl-aspartate (referenced to creatine), a marker of living neurons, in the cingulate (9). There is also evidence of decreases in glucose metabolism (10), cerebral blood flow (11), gray matter density (12), and decision-making-related activation in the ACC of individuals with methamphetamine abuse or dependence (13).

Tasks with unpredictable stop or nogo stimuli model scenarios involving an immediate, unexpected need to exert inhibitory control. However, many real world situations that require inhibition are preceded by environmental cues indicating that such control will or may be required in the near future. For instance, a red light in traffic signals the need to stop, but is usually preceded by a yellow light, which serves as an advance warning. Similarly, a “yield” sign signals the driver that he or she may need to stop if a vehicle with the right-of-way approaches. Cued go/nogo paradigms (e.g. 14,15) present stimuli that predict the need to respond/inhibit before go/nogo stimuli appear, improving both reaction time and inhibitory performance. Fillmore and Rush (16) have found that cocaine and alcohol polydrug abusers, like healthy comparison subjects, showed improved inhibition when presented with cues, although they had difficulty when the mapping of stimuli as go cues and nogo cues was switched. Such cued inhibition paradigms are particularly relevant to drug abuse research in that drug availability is frequently cued by specific people (e.g., a fellow user), places (e.g., a particular street corner), and events (e.g., a party) previously associated with a substance, even before the drug is immediately present for consumption. Successful inhibition thus depends not only on the ability to resist immediate prepotent urges but also on preparation for or avoidance of them by recognizing cues signaling their likelihood. Understanding the neural correlates of such cueing and its relationship to inhibitory control, therefore, may provide insight into how abstaining drug dependent individuals use warning cues to help them resist urges and avoid relapse.
To investigate this, we used a cued go/nogo paradigm (14) with methamphetamine dependent (MD) and healthy comparison (HC) subjects and focused on the effects of cues on the ACC. The ACC has been associated with error and conflict monitoring (17,18) and is activated in both go/nogo and stop-signal tasks (19). Brown and Braver (20) have suggested that the ACC subserves the learning of error likelihood in a given context, including errors associated with response conflict. They used a change-signal task in which a stimulus directing subjects to respond with a left versus right button press might (33% chance) be superseded by a subsequent stimulus requiring a response reversal. As in stop signal tasks, errors were more frequent when the delay between initial and subsequent stimuli was long. The color of the initial stimulus in this task indicated whether the delay would be short or long and thus served as a cue predictive of error likelihood. Cues predictive of high error likelihood activated the ACC more than cues predictive of low likelihood error. Magno et al. (21) recently demonstrated ACC activation when subjects rejected (i.e. they were allowed to “pass” on) difficult target detection trials in order to avoid making errors, consistent with the error-likelihood hypothesis.

These studies suggest that the ACC may not simply detect conflict but rather activate parametrically in response to the degree to which cues predict the need to inhibit a response. This, in turn, raises the question as to whether MD individuals are capable of using predictive cues to facilitate response inhibition and what relationship that ability has to ACC activation. We predicted that in-treatment abstinent MD patients performing a cued go/nogo task would show ACC activity to such cues, with signal intensity proportional to their beneficial effect on proper inhibition.

Methods and Materials

Participants

Nineteen MD individuals (17 male) and 19 age- and education-matched HC subjects (16 male) participated in the study, performing the go/nogo task during functional magnetic resonance imaging (fMRI). The MD participants were all treatment-seeking and met criteria for current dependence on methamphetamine as assessed by a psychiatrist, psychologist, or trained research assistant using the Structured Clinical Interview for DSM-IV (22). All MD individuals had voluntarily entered and completed a 28-day inpatient alcohol and drug treatment program at the San Diego Veterans Affairs Medical Center, which is based on a cognitive-behavioral model and includes a 12-step component with intense daily group sessions, education, and regularly scheduled Alcoholics Anonymous meetings. These inpatients are randomly tested for drugs and alcohol; any positive screen results in immediate discharge from the program. At the time of scanning, participants were abstinent from methamphetamine for 33.9 days (SD = 5.9; range, 25–50 days) and had used methamphetamine for 17.4 years (SD = 10.0; range, 4–38 years). HC subjects were recruited via advertisement in local newspapers; only those without a life-time history of Axis I disorders based on the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) were asked to participate in the study. In addition, subjects were excluded for significant head injury and neurological or other medical conditions compromising the central nervous system. The study was approved by the University of California, San Diego Human Research Protection Program. Each subject gave written informed consent and completed a postconsent questionnaire to demonstrate the capacity to provide informed consent.

Demographics

All subjects reported their age, gender, race/ethnicity, handedness, and years of education (Table 1). Eighteen participants from each group also completed the National Adult Reading Test (NART; 23) to provide an estimate of intelligence.

Experimental Paradigm

Participants viewed a serial presentation of blue shapes on a computer screen, all according to the same fixed stimulus sequence: 18 blocks of 10 trials each with baseline rest periods of 0–40 sec each between blocks. The stimuli consist of large circles (n = 64), small circles (n = 16), large squares (n = 43), and small squares (n = 57). Each stimulus appears for 200 msec.

| Table 1. Demographics, Methamphetamine Use, and Go/Nogo Performance Characteristics of Methamphetamine Dependent and Healthy Comparison Subjects |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Variable        | Meth-Dependent Subjects | Comparison Subjects |   |
|                 | Mean  | SD   | Range |       | Mean  | SD  | Range |   |
| Age (yrs)       | 40.4  | 9.9  | 24–54 | 40.3 | 8.1  | 26–56 | .97 |
| Education (yrs) | 13.4  | 1.2  | 12–16 | 14.0 | 1.6  | 12–16 | .17 |
| NART Errors     | 24.6  | 7.5  | 8–36  | 20.8 | 9.4  | 9–38  | .20 |
| Lifetime Use (yrs) | 17.4  | 10.0 | 4–38  |       |       |       |   |
| Sobriety (days) | 33.9  | 5.9  | 25–50 |       |       |       |   |
| Hit Rate        | .99   | .03  | .86–1.00 | .99  | .03  | .88–1.00 | .98 |
| False Alarm Rate | .11  | .09  | .00–.037 | .08  | .08  | .00–.28 | .43 |
| Beta            | .34   | .33  | .07–1.57 | .41  | .41  | .07–1.90 | .56 |
| d-prime         | 3.63  | .65  | 1.44–4.36 | 3.75  | .53  | 2.69–4.51 | .54 |
| Reaction Time (ms) | 821  | 106  | 689–1129 | 817  | 98  | 616–980 | .92 |
| Right-handed    | 17    | 89   |       | 17   | 89   |       | .60 |
| Caucasian       | 12    | 63   |       | 10   | 53   |       | .48 |
| Male            | 17    | 89   |       | 16   | 84   |       | .67 |

NART, National Adult Reading Test.

*p* test.

bChi-square test.
Subjects are instructed to press a button each time a large circle, small circle, or large square appears (go), and not to press the button when a small square is shown (nogo). The intertrial interval is 1500 msec.

Unbeknownst to subjects, the large square serves not only as an explicit go stimulus, but also as a predictive cue in that when it appears it is frequently (87% of the time) followed by a nogo trial. By comparison, small circles are never followed by a nogo and large circles are followed by a nogo only 30% of the time.

Behavioral Analysis

Between-groups demographic comparisons were made using unpaired t tests. To assess group differences in behavioral task measures, three t tests were run, each using one of the following dependent measures: hit rate, beta, and d-prime. To determine whether predictive cues facilitated proper inhibition, a 2 × 2 repeated measures analysis of variance was performed with false alarm (FA) rate as the dependent measure. Group (MD vs. HC) was the between-groups factor while Previous Trial (large square [cue] versus large circle [noncue]) was the within-subjects factor. Small circles were excluded from this analysis because they are never followed by Nogo stimuli, precluding the possibility of false alarms. A comparison of reaction times to each type of go stimulus was made using a 2 × 3 repeated measures analysis of variance with Group as the between subjects factor and go-type (large square, large circle, small circle) as the within-subjects factor.

Functional MRI Protocol and Image Analysis Pathway

Magnetic resonance images were obtained using a 1.5-T, whole-body system (Siemens, Erlangen, Germany). Anatomical T1-weighted images of the whole brain (magnetization prepared rapid acquisition gradient echo, repetition time = 11.4 msec, echo time = 4.4 msec, flip angle = 10°, field of view = 256 × 256, 1-mm3 voxels) were obtained sagittally. Thirty-two sections of T2-weighted images were obtained in the transverse plane (3.43 mm2). Functional images were acquired using an echo planar imaging preparation rapid acquisition gradient echo, repetition time of 3000 msec and an echo time of 43.2 msec, flip angle of 90°, 64 × 64-pixel field of view = 220 × 220 mm, 3-mm contiguous section thickness every 3000 msec for 112 (n = 19) or 128 (n = 27) repetitions yielding a voxel size of 3.43 mm3 × 3 mm, resampled to 4 × 4 × 4 mm voxels.

All data were preprocessed, normalized to Talairach coordinates (24) and analyzed with the Analysis of Functional Neuroimages (AFNI) software package (25). For preprocessing, voxel time series data were interpolated to correct for nonconsecutive slice acquisition within each volume and corrected for three-dimensional motion. Motion-corrected voxel time series data were visually inspected to remove large movement artifacts. Preprocessed time series data for each subject were analyzed using a multiple regression model consisting of nine regressors. Three regressors were used to model residual motion (in the roll, pitch, and yaw directions). Two regressors—a baseline and a linear trend—were used to eliminate slow signal drifts. There were four orthogonal regressors of interest: (1) large square (go, cue), (2) large circle (go, noncue), (3) small circle (go, noncue), (4) small square (nogo). These four regressors were created based on the sequence of stimulus events as described above and convolved with a model of a prototypical hemodynamic response prior to inclusion in the regression model. The AFNI program 3DDeconvolve was used to calculate the estimated voxel-wise impulse response function for each regressor and to generate two contrasts: Inhibition (nogo-go) and cueing (cue-noncue). The first contrast was used to compare activations for nogo and go trials (the latter collapsed across all three types), measure inhibition, and was created for comparison with previous go/nogo studies. The other contrast was created to examine the activation associated with predictive cues. Thus, it contrasted the go stimulus that served with high probability as a cue (large square) with those that did not (large circle, small circle). A Gaussian filter with full width half maximum 6.0 mm was applied to the voxel-wise percent signal change data to account for individual variations of anatomical landmarks.

Figure 1. Anterior cingulate cortex regions of interest (dorsal and ventral).

Two a priori regions of interest (ROIs) corresponding to the bilateral dorsal anterior cingulate cortex and the bilateral ventral anterior cingulate cortex were used as anatomical masks (see Figure 1). These ROIs were based on a combination of Talairach Daemon atlas definition (26) and the division of ACC into emotional and cognitive subregions as suggested by Bush et al. (27). We conducted Monte Carlo simulations using the AFNI AlphaSim program to guard against false positive activations. Based on these analyses, it was determined that a voxel-wise a priori probability of .05 would result in a corrected cluster-wise activation probability of .05 given a minimum volume of 448 µL or 7 connected voxels for the ventral ROI and 576 µL or 9 connected voxels for the dorsal ROI.

To investigate unpredicted regions of activation, we performed secondary whole-brain analyses for group effects on inhibition and cueing. Using a more conservative combined volume threshold method to prevent detection of false positive activations (cluster-wise p < .01 for each analysis), we found no significant differences.

Procedure

After consenting, participants completed interviews and NART testing. During a second visit, they were given task instructions and completed a practice go/nogo run immediately prior to the fMRI session to familiarize them with the task and control for confounding effects of learning from a task-naïve state during the scan session. Subjects performed the task during the fMRI session using button press responses. Before leaving, subjects were debriefed and paid for participating.
Results

Demographics
There were no significant differences in age, education, NART errors, handedness, race, or gender between the two groups (Table 1).

Task Performance
There were no differences between MD and HC groups on hit rate, beta, or d’ (Table 1). The groups did not differ on the number of false alarms ($F(1,36) = 1.90; p = .176$), but there was a trend toward an effect of Previous Trial ($F(1,36) = 3.63; p = .065$) and a significant Group × Previous Trial interaction ($F(1,36) = 4.59; p = .04$). Follow-up $t$-tests revealed that the FA rate was marginally lower when the previous trial was a cue ($0.08 \pm 0.07$) than when it was a noncue ($0.15 \pm 0.17$) in the MD group ($p = .028$, Bonferroni-adjusted $\alpha = .025$), whereas there was no difference between cued FA rate ($0.07 \pm 0.07$) and uncued FA rate ($0.07 \pm 0.09$) in the HC group ($p = .826$, Bonferroni-adjusted $\alpha = .025$). There was no main or interaction effect of Group on reaction time ($F < 1$) but there was a main effect of gotype, with pairwise comparisons revealing the following pattern: small circle > large square > large circle ($p < .001$).

Brain Activation
Each cluster is reported with volume, center of mass using Talairach coordinates (x, y, z) and $t$-value ($t$ test) or $r^2$ value (regression analysis). An all-subjects Inhibition (nogo-go) contrast revealed no significant activation in the ACC. Separate analyses revealed that each group demonstrated increased Inhibition-related ventral ACC activation, but in nonoverlapping areas. The cluster in the MD group (512 µL; x = 12, y = 34, z = 15; $t = 4.15$; Figure 2A) was superior, posterior, and to the right of that in the HC group (512 µL; x = 0, y = 46, z = 2; $t = 4.18$; Figure 2B).

MD subjects showed greater cueing-related (cue-noncue) activation than HC subjects in 2 ACC clusters (Figure 3), one in the ventral ROI (448 µL; x = −4, y = 37, z = 18; $t = 2.0$) and one in the dorsal ROI (896 µL; x = 0, y = 18, z = 29; $t = 3.6$). Separate within-group contrasts revealed no significant cueing effect anywhere in the ACC for HC subjects. MD subjects, on the other hand, showed significant activation in the ventral ROI in virtually the same place as shown in the group contrast (i.e., 576 µL; x = −7, y = 35, z = 21; $t = 3.6$). Similarly, there was an area of activation for MD subjects in the dorsal ROI that overlapped that found in the group contrast, but at 384 µL it did not reach the volume threshold for significance.

Using the same analysis pathway and thresholds described earlier, regression analyses were performed to determine whether the difference between cued and uncued FA rates seen in MD subjects was associated with their cueing-related (cue-noncue) activation in the ventral and/or dorsal ACC. An FA rate difference score was computed by subtracting the FA rate for uncued nogo trials (i.e., preceded by a noncue go trial) from the FA rate for cued nogo trials (i.e., preceded by a cue go trial): $FA_{cued} - FA_{uncued}$. A cluster in the ventral ACC was found (832

Figure 2. Anterior cingulate cortex activation in methamphetamine dependent (A) and HC (B) subjects during inhibition (nogo-go).

Figure 3. Cueing-related (cue-noncue) activation in anterior cingulate cortex in MD as compared with HC subjects (MD > HC).

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μL; x = −1, y = 45, z = 6; r² = .52) in which cueing-related activation intensity was negatively correlated with this difference score (Figure 4). That is, MD individuals with more cueing-related activation were those with the greatest cue-related drop in FA rate.

Discussion

There are two main findings of this investigation: first, MD subjects showed ACC activation in response to cues predicting the need to inhibit responses in a go/nogo task; second, the more MD individuals' ACCs were activated by such cues the better inhibitory performance was on trials following the cues. There was no cue-related ACC activation in HC subjects, possibly because cueing made no difference to their FA rate, which was low in both cases (~7%). Methamphetamine dependent subjects, on the other hand, had marginally fewer FAs to nogo following cues (7%) than noncues (15%). Methamphetamine dependent individuals, therefore, may have had inhibitory difficulties that were attenuated when they received advance warning, even though that warning was not given explicitly but rather was based on the predictive properties of a stimulus that itself requires action.

Cue-based ACC activation in this task is consistent with the error-likelihood hypothesis (20) to the extent that there was implicit learning of the probabilistic contingencies between cues and nogo, i.e. the potential for error in the form of inhibitory failure. This activation is also consistent with a response conflict interpretation in that large squares served two roles: 1) go stimulus, requiring immediate response, and 2) cue, signaling that the following trial would likely (87% chance) be a nogo, requiring inhibition of a prepotent response. This is supported by the finding that response latencies to large squares (cues) were longer than those to large circles (noncues), as found previously with this paradigm (14), perhaps reflecting response conflict given the cues’ dual role. Response latency was longest to small circles, which like large circles were noncues, but these were the least frequent stimuli and possibly the most visually similar to the small squares (nogo), which may have increased hesitation.

Cueing-related ACC activation may also reflect confounding task-specific factors. For instance, given that nogo stimuli are more likely to follow cues than noncues, some of the cue-Noncue difference may be due to overlapping Inhibition effects. This could be ruled out by comparing cue trials that are followed by nogo stimuli versus ones that are not, but in the current design there are too few of the latter to permit such an analysis. While a high nogo prediction rate is necessary to create effective cues, future work can address this limitation by increasing the number of trials or by lengthening the intertrial interval to minimize potential overlap. Another potential confound comes from differences in presentation rate for the different go stimuli. Braver et al. (28) have demonstrated increased ACC activation to low frequency events independent of task demands. In this task, Large squares (cues) are less frequent than large circles (non-cues) but more frequent than small circles (noncues). Thus, it is unclear how stimulus probability would influence the cue-noncue contrast. It seems unlikely, however, that the Cueing effect is entirely a byproduct of presentation rate differences, given the observed correlation between cueing effects on ACC activation and false alarm rates in MD individuals.

Methamphetamine dependent subjects in this study performed on par with HC subjects and both showed more ACC activation during inhibitory trials than go trials. This is in contrast to previous findings of impaired go/nogo inhibition and cingulate hypoactivity in cocaine users (6,7), as well as findings of impaired inhibitory performance by MD individuals on a stop-signal task (4). One possible explanation is that this version of the go/nogo task employed a large number of blocks with few trials in each, which may have decreased working memory demands relative to other versions, making it easier to perform and less likely to reveal a difference in performance and brain activity relative to HC subjects. Another noteworthy difference is that the MD participants in this study were not active users but inpatients receiving voluntary treatment.
warned of them by environmental cues, and for better recognizing such cues in the first place. Compared with initial assessments, posttreatment testing might provide an index of the success of such interventions in improving the cognitive skills necessary for sustained remission from methamphetamine dependence.

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