

# THE INFLUENCE OF GENETIC VARIATION ON THE TIME COURSE OF ANTERIOR CINGULATE ACTIVATION DURING CONFLICT AND ERROR DETECTION

Jin Fan\*, John Fossella\*, Michael S. Worden\*, Yanghong Wu‡, Tobias Sommer\*†, Bruce D. McCandliss\*, and Michael I. Posner §\*

\*Sackler Institute for Developmental Psychology, Weill Medical College of Cornell University, New York, NY 10021; † Department of Neurology, University of Hamburg, Germany; ‡ Department of Psychology, Peking University, China, §Department of Psychology, University of Oregon, Eugene, OR 97403;



## Introduction

We previously found polymorphisms in the dopamine D4 receptor gene (DRD4) and monoamine oxidase a (MAOA) gene were related to conflict as measured by RT in the Attention Network Test (ANT) (Fossella et al, 2002) and these genes were related to different levels and locations of activation in the anterior cingulate in an fMRI study (Fan et al 2003).

We now report a high density (128 channel) event-related electrical potential (ERP) study designed to explore the time course of activation of the cingulate during conflict and error detection. Differences between incongruent and congruent targets allow us to examine the time course of conflict resolution. Contrasting correct and error trials can be used to examine the time course of the error related negativity (ERN).

To examine whether these genetic variations might contribute to differences in the time course of brain activation, ERP subjects were genotyped for the DRD4 and MAOA genes.

## Method

**Participants** Sixteen right-handed normal adults (mean age = 27.5 years, SD = 6.2, range:20-36 years; 8 female) participated in this study.

**Stimuli and task** For the flanker task, stimuli consisted of a row of 5 visually presented horizontal black lines, with arrowheads pointing leftward or rightward, against a gray background. The target was a leftward or rightward arrowhead at the center. This target was flanked on either side by two arrows in the same direction (congruent condition), or in the opposite direction (incongruent condition). The participants' task is to identify the direction of the centrally presented arrow by pressing one key for the left direction and a second key for the right direction.

**EEG acquisition** EEG was recorded from 128 scalp sites using the 128 channel Geodesic Sensor Net. All recordings were initially referenced to Cz during recording, and later digitally re-referenced using an average reference transformation. EEG was recorded using a 0.1 – 100 Hz band-pass.

**Source analysis** For each subject, two sets of difference waves were calculated. A target-locked measure related to conflict was calculated by subtracting congruent from incongruent trials for which there was a correct response. Response locked potentials for correct and incorrect responses on incongruent trials were subtracted to calculate the ERN. These difference waves were submitted to the BESA (Brain Electric Source Analysis) for the source analysis. The analysis of the generators was guided by the location of activations in the previous fMRI study. Figure 2 shows the coordinates for the source modeling based on the fMRI study.

**Genotyping analysis** Buccal swabs were obtained via buccal cell brush from consenting subjects. Yields range from 0.5 to 3 µg of DNA from each buccal sample. Yields were determined spectrophotometrically by absorbance at 260nm. Taq polymerase, PCR buffer and dNTPs were obtained from QIAGEN and used at recommended concentrations for a 20µl PCR reaction. PCR reactions and restriction digests (PCR-RFLP) are optimized for each marker. A 'touchdown' PCR cycling regimen and the addition of DMSO (10% final v/v) was used. Gel electrophoresis in either LE agarose followed by staining in ethidium bromide was used to resolve and visualize DNA fragments. For genotyping of the MAOA-LPR, Forward: 5'-ACAGCCTGACCCGTGGAGAAG-3' and Reverse 5'-GAACGGACGCTCCATTCGGA-3' primers were used. The insertion/deletion of a guanosine or 'G' residue at upstream position -1217 was genotyped using forward 5'-TGACAAGAGGACTGAGCCTGGCT-3' and reverse 5'-GGCGGCACATCTGATGCTCTAGT-3' followed by digestion with BstEII.

## Results

**Behavioral results** Mean RT for correct trials was 509 ms for incongruent trials and 455 ms for congruent trials. For error trials, 432 ms for incongruent and 412 ms for congruent. The conflict effect was 54 ms (correct incongruent – correct congruent). For MAOA, 4-repeat group was faster than 3-repeat on RT,  $F(1,14)=5.582, p < .05$ . However, for DRD4, the two groups (insertion-allele-class and deletion-allele-class) were not different on RT,  $F < 1$ .

	Congruent	Congruent error	Incongruent	Incongruent error
MAOA				
4-repeat (n=11)	437	386	481	407
3-repeat (n=5)	471	456	542	475
DRD4				
Insertion (n=6)	450	416	508	442
Deletion (n=10)	446	403	495	420
Mean (n=16)	455	412	509	432

**ERP results** For the stimulus locked ERPs, the waveforms for the four categories: congruent, congruent error, incongruent, incongruent error, and the difference between correct incongruent and congruent averages are shown in Figure 1. Differences between the incongruent and congruent conditions begin at about 300 msec. This is consistent with previous results (Van Veen & Carter, 2002). Differences between correct and error trials arise mostly from the incongruent condition and differ immediately (0 msec) after the response. This is somewhat earlier than in previous studies.

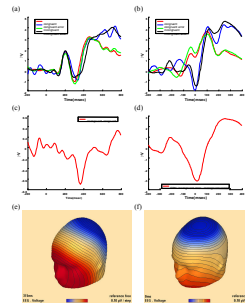


Figure 1. ERPs from midline channel 6 demonstrating the onset of the four conditions. (a) target locked ERP; (b) response locked ERP. (c) conflict effect (incongruent minus congruent, correct trials only). (d) ERN (incongruent error minus incongruent correct). (e) and (f) 3D voltage maps corresponding to (e) conflict and (f) ERN.

**Source modeling** Figure 2 shows coordinates for the source modeling. The dipole locations were fixed with the orientations free. Source modeling was conducted subject by subject. The ERP waves were averaged for each group. Figures 3-5 show results of source modeling for the conflict effect and ERN as a function of allele

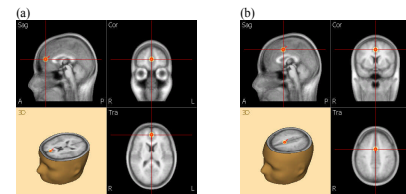


Figure 2. Coordinates for the source modeling based on the fMRI study. The dipole locations are fixed with the orientations free. (a) For MAOA groups (0, 47, 14); (b) For DRD4 groups (0, 5.8, 38).

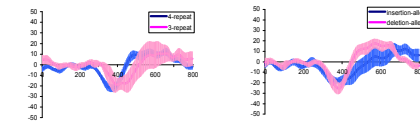


Figure 3. Dipole modeling for MAOA (left) and DRD4 (right) groups on the difference between incongruent and congruent (target locked). The conflict difference wave demonstrated an effect 296ms after target onset when contrasting the 4-repeat and 3-repeat groups of MAOA gene.

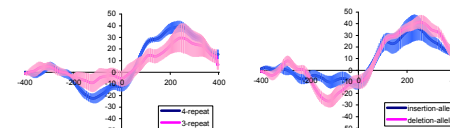


Figure 4. Dipole modeling for MAOA (left) and DRD4 (right) groups on ERN (the difference between incongruent error and incongruent correct).

## Discussion

The results indicate that polymorphisms in one of the two genes studied (MAOA) may be related to differences in conflict processing as measured by both RT and conflict related differences in the ERP (onset and amplitude). These differences occur at about 300 ms after input. MAOA has effects on dopamine, norepinephrine and serotonin transmitter systems. The genetic differences we report seem specific to the conflict operation since they are not found for the ERN. This finding raises the possibility that individual differences in the conflict and ERN effects have at least partly different origins.

In this study we were not able to replicate differences in the DRD4 gene that we reported previously. This may result from the small number of subjects in the current study.

The overall results support our previous efforts to understand the influence of genes on the development of neural networks underlying attention. EEG can be used to supplement the cognitive tasks and fMRI methods that we have used previously to explore such questions and relate to the amount of activation of the executive network, as assessed by the ANT fMRI study, specifically the anterior cingulate gyrus. A similar analysis of the ERN revealed no relation between its amplitude in our ERP study and these same genes, suggesting that individual differences in conflict and error monitoring have at least partly different origins.

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Please send email to Jin Fan: [jif2004@med.cornell.edu](mailto:jif2004@med.cornell.edu) or download from [www.sacklerinstitute.org/~jinfan](http://www.sacklerinstitute.org/~jinfan)