Possible association between response inhibition and a variant in the brain-expressed tryptophan hydroxylase-2 gene

Scott F. Stoltenberg  
University of Nebraska-Lincoln, sstoltenberg2@unl.edu

Jennifer M. Glass  
University of Michigan

Steven T. Chermack  
University of Michigan

Heather A. Flynn  
University of Michigan

Sheng Li  
University of Michigan

See next page for additional authors

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The ability to inhibit responses is critical for normal behavioral flexibility (Logan et al., 1984) and to direct behavior to achieve long-term goals (Nigg, 2000). Deficits in response inhibition are associated with several psychiatric disorders, such as attention deficit/hyperactivity disorder (ADHD) (Schachar et al., 2000), and substance abuse (Vogel-Sprott et al., 2001; Fillmore and Rush, 2002).

The serotonin system influences impulsivity in human beings and in other animals (Linnoina et al., 1983; Westergaard et al., 2003; Winstanley et al., 2003; Clarke et al., 2004). Impulsive aggression decreases with the administration of drugs that raise serotonin levels (New et al., 2004), and impulsivity is increased by diets that reduce serotonin levels (Crean et al., 2002; Walderhaug et al., 2002). Studies have reported associations between serotonin system gene variants and ADHD (Hawi et al., 2002; Sheehan et al., 2005), impulsivity and suicidality in major depressive disorder (Arango et al., 2001; Mann et al., 2001) and antisocial alcoholism (Hill et al., 2002). Response inhibition has been localized to the prefrontal cortex (Aron and Poldrack, 2005), which is innervated by serotonergic neurons. No studies to date have reported using a laboratory measure of response inhibition to examine potential associations with serotonergic genetic markers.

The University of Michigan Medical School Institutional Review Board approved this study. One hundred and ninety-nine participants (111 females, 88 males) (a) responded to advertisements offering $50 for 90 minutes of anonymous participation, (b) completed self-report questionnaires, (c) completed the computerized Stop Task, and (d) donated buccal cells. Their age ranged from 18 to 38 years (mean=20.8). Data for these analyses included the 67.8% (N= 135) self-identified as “White, not of Hispanic origin” (to control for population stratification). For multivariate analyses, only cases without missing data were included (final N = 109, 67 females; 42 males).

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Scott F. Stoltenberg,1,2 Jennifer M. Glass,2 Steven T. Chermack,2 Heather A. Flynn,2 Sheng Li,3 Margaret E. Weston,2 and Margit Burmeister 3

1. Department of Psychology, Black Hills State University, Spearfish, South Dakota
2. Addiction Research Center, Department of Psychiatry, University of Michigan, Ann Arbor, Michigan
3. Mental Health Research Institute, Department of Psychiatry, University of Michigan, Ann Arbor, Michigan

Corresponding author — S. F. Stoltenberg

Abstract
The ability to inhibit a response is an important component of normal behavioral control and is an aspect of psychopathology when diminished. Converging evidence implicates the serotonergic neurotransmitter system in response inhibition circuitry.

Objectives — The present study examined potential associations between serotonergic genetic markers and response inhibition as indexed by Stop Task performance.

Methods — College-age participants (N= 199) completed self-report questionnaires, the computerized Stop Task, and donated buccal cells for genetic analyses. Statistics were analyzed by ANOVA.

Results — Stop Signal reaction time was not associated with allelic variation at a monoamine oxidase A promoter length polymorphism or a serotonin 1B terminal autoreceptor polymorphism (G861C). An intronic genetic marker of the neuronal tryptophan hydroxylase-2 (the rate-limiting enzyme for serotonin biosynthesis) gene, however, was associated with the Stop Signal reaction time. Individuals homozygous for the T variant at an intron-8 polymorphism had the longest Stop Signal reaction time (i.e. greater impulsivity, P = 0.01), and this effect was stronger in males (P = 0.01) than in females (P = 0.10).

Conclusions — A genotype at an intron-8 tryptophan hydroxylase-2 polymorphism was associated with response inhibition as indexed by the Stop Task. These results, if replicated, would implicate dorsal raphe serotonin neurons in response inhibition. It may be that individuals with the T/T genotype may have reduced tryptophan hydroxylase-2 function and correspondingly lower central serotonin levels; however, further investigation of the reported association is required.

Keywords: serotonin, association study, impulsivity, stop task
For the Stop Task, an “X” or an “O” is displayed on a computer screen, and instructions are to respond rapidly by pressing one of two keys, depending on the visual stimulus. On 25% of trials, a tone [Stop Signal (SS)] sounds after the X or O appears, indicating that participants should withhold responding. The delay between the visual stimulus and the SS starts at 250 ms and is dynamically adjusted depending on the participant’s response. If a response was made, the delay for the next SS decreased by 50 ms (easier to inhibit); if no response was made, the delay for the next SS was similarly increased (harder to inhibit). This delay yields a response inhibition rate of approximately 50% and provides reliable estimates of StopRT (Band et al., 2003). To calculate StopRT (an index of inhibitory control), average SS delay is subtracted from average GoRT. StopRT is related to normal variations in impulsivity (Logan et al., 1997) and to differences in behavioral inhibition in various psychopathologies (Nigg, 1999; Fi themore and Rush, 2002; Nigg et al., 2002, 2004). Thus, the StopRT is useful for understanding both normal and impaired executive function.

In all, there were 64 practice trials, followed by four blocks of 80 trials. We averaged the final four blocks unless, during a block, a participant inhibited responding on less than 30% or more than 70% of trials, which was taken as a failure to follow instructions. Data from only those “bad” blocks were discarded and the participant’s mean scores were based on the remaining blocks (Nigg, 1999). Data from 15 participants were discarded in this manner.

Average GoRT on trials without an SS was 483.40 ms. Males (459.24 ms) were faster, on average, than females [506.31 ms; \( F_{(1,107)} = 4.43, P = 0.04 \)]. Average StopRT was 225.39 ms (males, 232.18 ms; females, 221.13 ms, NS).

DNA extracted from buccal cells (Puregene Kit, Gen tral Systems, Minneapolis, Minnesota, USA) was used in polymerase chain reactions (PCRs) carried out in 20 ml volume, using 2 μl genomic DNA, 200 μM of each dNTP, and 1.5 U Taq polymerase. PCR products were separated by electrophoresis in agarose gels and visualized using 5.0 U HincII (New England Biolabs, Ipswich, Massachusetts, USA).

MAOA is an enzyme that inactivates biogenic amines throughout the body (Xp11.23). Variants in the MAO promoter region and in the gene have been associated with impulsivity and aggression (Manuck et al., 2000). For the VNTR polymorphism in the monoamine oxidase A (MAOA) gene promoter region PCR, the following primers were used: MaoaPT1 5′ ACA GCC TGA CCG TGG AGA AG and MaoaPB1 5′ GAA CCG ACG TCT CAT TCG GA. Amplification was carried out with 20 pmol of each primer in buffer [167 mM (NH4)2SO4, 670 mM Tris pH 8.8, 20 mM MgCl2, 100 mM 2-mercaptoethanol, 10 mg/ml bovine serum albumin], 1.2 M betaine, and 1% dimethylsulfoxide for 35 cycles (1 min at 94°C, 2 min at 55°C, and 3 min at 72°C, with a final extension of 10 min at 72°C).

Tryptophan hydroxylase is the rate-limiting enzyme in serotonin synthesis. Association of variants in an intron and in the promoter of tryptophan hydroxylase (TPH) have been found with impulsive-aggressive behavior, suicidality, ADHD, and depression-related traits (Nielsen et al., 1998; Tsai et al., 1999; Turecki et al., 2001; Sheehan et al., 2005). Recently, tryptophan hydroxylase-2 (TPH2) (12q21.1) was found to be the main gene encoding TPH in the brain (Walther and Bader, 2003). For the intron-8 polymorphism (rs1386483) of TPH2 PCR, the following primers were designed: TPH2f-5′ GCT GCC TCT GAA CGT GTA TTT TG and TPH2r-5′ GAA GCC TGA CCG GTA TTT. Amplification was carried out with 20 pmol of each primer in buffer [66 mM Tris pH 9.0, 1.5 mM MgCl2] for 35 cycles (1 min at 95°C, 2 min at 53°C, and 3 min at 72°C), and products were digested using 2.5 U HinII (New England Biolabs).

Allele frequencies were calculated by pooling across sex, except for MAOA, which is located on the X chromosome and therefore was examined separately by sex. For 5-HT1B G861C, G = 0.79, C = 0.21 (N = 52); for MAOA VNTR, males 1 = 0.48, 3 = 0.52 (N = 29), females 0 = 0.01, 1 = 0.36, 3 = 0.63, 4 = 0.01 (N=52); for TPH2, T = 0.34, C = 0.66 (N=119). The markers were in Hardy–Weinberg equilibrium. Sample sizes varied because of difficulties in amplifying some samples.

ANOVA/WS with Sex and Genotype as fixed effects and StopRT as the dependent variable were not significant for 5-HT1B and MAOA. For TPH2 (see Figure 1), both
(which is not in HAPMAP) was recently reported to be associated with depression and SSRI response (Zhang et al., 2005). It is worth noting that substantial linkage disequilibrium has been found along the TPH2 gene (Zill et al., 2004). Future research may address whether the T variant of the intron-8 polymorphism in TPH2 that we found associated with StopRT is in linkage disequilibrium with the novel reduced function TPH2 variant. Our finding appears to be the first reported association between the brain-expressed TPH2 gene and a laboratory measure of impulsivity.

This finding adds to the converging evidence that TPH2 is associated with impulsivity. Our result, however, like all first reports on genetic association studies, does not provide sufficient evidence to support a causal role for TPH2 in impulsivity, given the potential for false positives. Further research is needed to replicate this finding and to investigate the biological mechanisms underlying the association between TPH2 and impulsivity.

References


