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Serotonin transporter polymorphisms predict response inhibition in healthy volunteers

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HIGHLIGHTS

- 5-HTTLPR polymorphisms mediate different levels of inhibitory control.
- More impulsive behavior in healthy carriers of the low expressive genotype.
- These initial observations will allow extensions to patient populations.

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ABSTRACT

Serotoninergic transmission is reliably implicated in inhibitory control processes. The aim of this study was to test the hypothesis if serotonin transporter polymorphisms mediate inhibitory control in healthy people. 141 healthy subjects, carefully screened for previous and current psychopathology, were genotyped for the 5-HTTLPR and rs25531 polymorphisms. Inhibitory control was ascertained with the Stop Signal Task (SST) from the Cambridge Neuropsychological Test Automated Battery (CANTAB). The triallelic gene model, reclassified and presented in a biallelic functional model, revealed a dose-dependent gene effect on SST performance with Individuals carrying the low expressive allele had inferior inhibitory control compared to high expressive carriers. This directly implicates serotonin transporter polymorphisms (5-HTTLPR plus rs25531) in response inhibition in healthy subjects.

1. Introduction

Impulsivity can be understood as arising from impairment in inhibitory control [1]. Impaired serotonin (5-HT) function [2] has been shown to contribute to the neurobiology of impaired executive control processes [3] and impulsive behaviors [4]. However, the genetic contribution of these behavioral processes is incompletely understood.

The 5-HT-transporter-linked polymorphic region (5-HTTLPR) in the promoter region of the human 5-HT transporter (5-HTT) gene (SLC6A4) results in two main alleles or variants [5]; the short (S) and long (L), comprising 14 and 16 copies of a 20–23 nucleotide repeat cassettes, respectively. A functional triallelic 5-HTTLPR polymorphism include an additional single nucleotide polymorphism A > G SNP (rs25531) in the first of two 22-bp imperfect repeats that define the 16-repeat L allele. The 5-HTTLPR L allele combined with the major allele A in rs25531 (L_A) is associated with higher expression of the transporter protein compared to the L_G allele and the short S allele [6], resulting in altered 5-HT tone and neurotransmission.

Few studies have directly studied the potential role of the 5-HTTLPR polymorphisms and inhibitory control in healthy subjects under laboratory conditions and results so far are conflicting. Whereas some studies found no association between 5-HTTLPR variants and measures of inhibitory control and impulsivity [7,8], others reported that the short allele of the 5-HTTLPR may mediate impairments in impulse control [9,10]. Operationalization of inhibitory control varies across studies, with three of the four studies using variants of continuous performance/go-no go tasks, and another study applying the Stop Signal Task [8], which requires

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the cancelation of a motor response that has already been initiated [11]. The Stop Signal Task offers significant psychometric advantages over conventional Continuous Performance, or Go/No Go Task, since the difficulty of stopping can be adjusted for each individual by manipulating the delay between the Go stimulus and the stop signal.

The aim of this study was to test the potential role of the 5-HTTLPR polymorphism in mediating inhibitory control in healthy people, specifically if carriers of the low expressive 5-HT transporter variant (5-HTTLPR S and L_G) exhibit less effective inhibitory control relative to carriers of the high expressive variant (L_A).

2. Methods

157 healthy subjects (105 females, 52 males) were recruited from the general public using advertisements in a local newspaper in Oslo. Mean age of the cohort was 36.4 years (SD = 13.1), ranging from 19 to 64 years of age. After giving written informed consent, the participants provided information about their medical status and underwent psychiatric evaluation including the Diagnostic Interview for Genetic Studies [12], the Structural Clinical Interview for DSM-IV, Axis I and II disorders (SCID I and SCID II). Depression and anxiety symptoms were assessed using the Beck Depression Inventory [13] and the Beck Anxiety Inventory [14], respectively. The SCID interviews were administered and recorded by trained clinicians and were subjected to consensus diagnoses. Subjects fulfilling the criteria of any psychiatric diagnosis were excluded, including subjects with current/ongoing drug abuse or dependency. Other exclusion criteria were head trauma during the last year with loss of consciousness greater than 30 min as well as other neurological disorders. Education level was classified by means of the International Standard Classification of Education [15]. General cognitive functioning was estimated from scaled scores of two subtests from the WAIS-III, Picture Completion and Similarities [16]. The subjects were given a $ 50 gift certificate for their participation. The Regional Ethics Committee approved the project.

2.1. Genotyping

The biallelic 5-HTTLPR polymorphism, located in the regulatory region of the 5-HT transporter gene (SLC6A4), was genotyped essentially as described in detail elsewhere [17,18]. A real-time fluorescence Light Cycler instrument was used to amplify genomic DNA by polymerase chain reaction (PCR) in a final volume of 20 μL using Light Cycler Faststart DNA SYBR Green kit (Roche cat no. 12239264001) with specific primers (0.5 μM) [17] generating a long (L) 419 base pair (bp) or a short (S) 375 bp PCR product. Differences in product length depend on the variable number of a 22 bp tandem repeat (VNTR) sequence in the promoter region. Cycle conditions were initiated by 10 min denaturation (95 °C) followed by 45 cycles at 95 °C (10 s), 66 °C (10 s) and 72 °C (10 s). For the detection of the additional A > G SNP (rs25531), the PCR fragments were digested with 1 U MspI restriction enzyme (New England Biolabs, Beverly, Massachusetts) for 2 h at 37 °C. The PCR fragments contain two obligatory MspI sites, whereas the A > G substitution creates an additional MspI site. The PCR reaction followed by restriction digestion and gel electrophoreses provides classification of the S, L_A and L_G alleles.

2.2. Inhibitory control measure (the Stop Signal Task; SST)

The Stop Signal Task was selected from the Cambridge Neuropsychological Test Automated Battery [19]. Trained research assistants administered the SST. This task measures the ability to inhibit an already-initiated motor response [11]. In a subset of trials (i.e. 25%), an auditory beep occurs (the "stop signal") to indicate that the response should be withheld on that particular trial. A procedure is applied to track the participants' performance, by varying the stop signal delay (SSD) parameter after successful and unsuccessful stop attempts. Over time, this tracking procedure stabilizes the probability of successful inhibition around 0.5 for each subject. The Stop signal reaction time (SSRT), calculated by subtracting the SSD_{50} from the median Go RT, is the main outcome variable. Thus, the SSRT reflects the effectiveness in the ability to inhibit a prepotent response. The total number of Go discrimination errors (i.e. a right button press to a left-facing arrow) was also registered.

Sixteen subjects failed to achieve convergence, either through too high (≤60%) or too low (≤40%) levels of successful inhibition. These staircase failures may arise through strategic slowing of the go reaction time, or through inconsistent performance or excessive distraction. They invalidate an assumption of the horse race model that Go-and stop-related processes are independent [11]. Thus, the final group for analysis was a total of 141 participants (94 females, 47 males).

3. Statistical analyses

One-way ANOVAs were conducted to explore possible group differences in age, education level (ISCED) and general cognitive functioning (sub-tests Similarities and Picture Completion from WAIS-III), as well as symptoms related to depression (BDI) and anxiety (BAI). A one-way ANOVA was conducted to predict Stop Signal Task performance from 5-HTTLPR genotype combined with the A > G SNP (rs25531). Polynomial contrast was performed to test the dose effects across genotypes. Levene's test of homogeneity confirmed that the groups were not significantly different in variance, thus validating use of the F test. Finally, a linear regression model was conducted to explore the amount of unique variance explained by genotype after taking variance explained by group differences (from the ANOVAs) into account.

4. Results

The triallelic classification was reclassified into a functional model, based on the 5-HTTLPR-directed level of transcriptional activity of the transporter gene as follows: L_G/S, L_G/L_G and S/S genotypes were classified as SS' (low leveled RNA transcription); L_A/S and L_A/L_G genotypes were classified as LS' (intermediate leveled); and L_A/L_A genotype was classified as LL' (high leveled) [20].

The genotype distribution was L_AL_A 22.9%, L_GL_A 10.2%, L_AS 39.5%, L_GL_G 0.6%, SL_G 7.6% and SS 19.1%. The genotype distribution was in Hardy-Weinberg equilibrium ($x^2 = 0$, $df = 1$; $p = 0.99$). The genotype distribution after exclusion ($n = 141$) was L_AL_A 24.1%, L_GL_A 9.9%, L_AS 41.1%, L_GL_G 0.7%, SL_G 7.8% and SS 16.3%.

There was a significant difference between the genotype groups in education level ($F(2, 138) = 4.168$, $p = 0.017$, $\eta^2 = 0.057$) and a trending toward significant difference in age ($F(2, 138) = 3.006$, $p = 0.053$, $\eta^2 = 0.042$). There were no statistically significant differences between 5-HTTLPR sub-groups on BDI, BAI, or the two subtests from WAIS. Education level and age was therefore added in the final regression model. There was no statistical significant sex by genotype interactions for any of the SST variables. Because of this, the analyses were collapsed across gender.

There was a statistically significant effect of 5-HTTLPR plus A > G SNP on the SSRT variable ($F(2, 138) = 3.518$, $p = 0.032$, $\eta^2 = 0.049$). Polynomial contrast measure revealed a linear effect of the number of low expressive alleles (CE = 21.6, $p = 0.009$) (Fig. 1). SSRT was predicted by 5-HTTLPR genotype (Beta = 0.195, $p = 0.022$), but not by age (Beta = 0.157, $p = 0.65$) or education level (Beta = −0.030,