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HTTLPR functional polymorphism in schizophrenia: Executive functions vs. sustained attention dissociation

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ABSTRACT

Background: Recently attention has been addressed to the role of 5-HT in cognition and several experimental studies revealed that manipulations of the central 5-HT system can produce quite specific changes in cognitive functioning. These results may suggest new treatment strategies to improve cognition in psychiatric conditions characterized by neuropsychological impairments, such as schizophrenia. It is possible to investigate the involvement of 5-HT in cognition by examining the impact of genetic variation in key regulators of serotoninergic neurotransmission. Among these, the serotonin transporter (5-HTT) presents a functional polymorphism in the transcriptional control region of the gene (5-HTTLPR) affecting transcriptional efficiency. In the present study, we aimed to analyze the effect of 5-HTTLPR polymorphism on specific cognitive functions, known to be affected by 5-HT manipulation and altered in schizophrenia.
Methods: 223 schizophrenia patients were tested with Wisconsin Card Sorting Test (WCST), for the evaluation of cognitive flexibility, Continuous Performance Test (CPT), for the evaluation of attention, and genotyped for the 5-HTTLPR.
Results: We found a significant association between HTT polymorphism and executive functions and inversely with sustained attention. The presence of the high-activity long (L) allele in homozygosis was a predictor of better executive performances and poorer performances of attention.
Conclusions: Our findings suggest that factors affecting serotonin availability may play a specific role in cognitive processes, probably through complex modulation of the different performance components.

1. Introduction

In the past decade, experimental works in humans have revealed that pharmacological manipulations of central serotonin (5-HT) system can produce specific changes in cognitive functioning (Goldman-Rakic, 1999), notably in attention and executive functions.

Regarding attentional functions, there is evidence in populations of healthy men and women that acute tryptophan depletion (ATD) improves performances of focused attention for neutral stimuli (Schmitt et al., 2000) and that the changes are dose-dependent (Booij et al., 2005). Moreover functional magnetic resonance imaging (fMRI) studies observed increased activation of anterior cingulate cortex during performance of a focused-attention task in healthy females after ATD (Evers et al., 2006). Several studies also showed that augmentation of 5-HT neurotransmission by administration of selective serotonin reuptake inhibitors (SSRIs) impairs human vigilance performances in healthy volunteers of both sexes. A reduction of sustained attention was observed after subchronic treatment with fluoxetine 20 mg (Ramaekers et al., 1995), paroxetine 20–40 mg (Schmitt et al., 2001) and citalopram 20–40 mg (Riedel et al., 2005). A recent work of fMRI in a sample of 5 males and 5 females (Wingen et al., 2008) reported a decreased activation in several brain areas, including the basal ganglia and the frontal cortex, after escitalopram administration, suggesting that increments in serotonin levels may impair sustained attention through influencing the interaction between the cortical and subcortical networks involved in arousal and attention.

Data from animal and human studies suggest that 5-HT is also involved in modulation of cognitive flexibility and the effect seems to

Abbreviations: ANOVA, analysis of variance; ANCOVA, analysis of covariance; ATD, acute tryptophan depletion; CPT, continuous performance test; ERPs, event-related potentials; fMRI, functional magnetic resonance imaging; 5-HT, serotonin; 5-HTT, serotonin transporter; PANSS, positive and negative syndrome scale; SSRIs, selective serotonin reuptake inhibitors; WCST, Wisconsin card sorting test.

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be in the opposite direction compared to attention. Animal research showed that low 5-HT leads to inflexible behaviour (Barnes et al., 1990; Harrison et al., 1999) and studies on healthy volunteers supported this result observing impairment in cognitive flexibility after ATD in females (Murphy et al., 2002) and also in a sample balanced for gender (Rogers et al., 2003). Among the few fMRI studies investigating the ATD effect on brain activation while performing a cognitive flexibility task, Evers et al. (2005) observed increased activation in the dorsomedial prefrontal cortex related to negative feedback in male healthy volunteers.

Given these evidences from 5-HT pharmacological manipulation studies, it appears of great interest to investigate the influence of genetically determined variability in serotoninergic gene expression on human cognition. 5-HT acts as a master control neurotransmitter within a highly complex system of neural communication mediated by multiple pre and post synaptic 5-HT receptor subtypes, while high affinity 5-HT transport into the presynaptic neuron is mediated by a single protein complex, the serotonin transporter (5-HTT), thus playing a pivotal role in the fine-tuning of 5-HT transmission (Lesch et al., 1997). The transcriptional activity of the 5-HTT gene on chromosome 17q11.2, is modulated by a polymorphic repetitive element (5-HTT gene-linked polymorphic region, 5-HTTLPR), located upstream of the transcription start site: cell homozygous for the long variant (L) produce higher concentrations of 5-HTT mRNA leading to more than 2-fold higher rate of 5-HT uptake than cells containing one or two copies of the short (S) form (Lesch et al., 1996). The downstream effect of this polymorphism on 5-HT transmission and moreover on 5-HT related behaviours and cognitive domains is still poorly understood, and the relatively few studies exploring the role of 5-HTTLPR polymorphism on cognition showed contradictory results (Borg et al., 2009; Da Rocha et al., 2008; Izquierdo et al., 2007) .

The imbalance of serotoninergic transmission has been implicated both in the pathophysiological processes of schizophrenia (Sawa and Snyder, 2002) and in the antipsychotic mechanism of action (Meltzer and Huang, 2008). Particularly, the serotonin transporter (5-HTT) appears to be involved in the development of schizophrenia, since post-mortem studies have reported reduced serotonin reuptake sites in the brains of schizophrenic patients (Naylor et al., 1996) and an increase in 5-HTT mRNA levels in the cortex of schizophrenic individuals (Hernandez and Sokolov, 1997). For these reasons the serotonin transporter gene has been extensively studied in schizophrenia for association with the disorder's susceptibility (Kaiser et al., 2001; Zaboli et al., 2008), with clinical symptomatology (Malhotra et al., 1998; Pae et al., 2006; Tsai et al., 2000) and with antipsychotic response (Lee et al., 2009), but the results reported are still conflicting. However to our knowledge there are no studies investigating the association between the serotonin transporter gene polymorphism and cognitive functions in schizophrenia. Among core neuropsychological deficits observed in schizophrenia patients, impairments of attentional and executive functions are the most frequently reported (Ermoli et al., 2005)) and correlate with poor global functional outcome and social disability (Green et al., 2000).

Many evidences from 5-HT manipulation studies in healthy volunteers proved a critical influence of this neurotransmitter in specific cognitive functions, particularly in attention and executive functions, which seem to be inversely modulated by serotonin concentrations (low 5-HT is mostly associated with improved attention and impaired cognitive flexibility), while the role of genetically driven variability of 5-HTT function on cognition is still unclear. In this view we investigated the effect of the 5-HTTLPR on attention and executive functions in a sample of patients affected by schizophrenia, hypothesizing that in a clinical population characterized by core impairment of these domains and suggested imbalance of serotoninergic transmission, genetically determined alterations in 5-HT uptake could influence performances in greater measure than in healthy subjects.

2. Methods

2.1. Sample

The study group included 223 biologically unrelated outpatients. Inclusion criteria were: diagnosis of schizophrenia meeting DSM-IV criteria, age from 18 to 65 years, treatment with a stable dose of the same antipsychotic in monotherapy since at least 3 months, and good response to treatment (defined as a reduction of 30% or more in PANSS Total Score after 3 months of treatment). Exclusion criteria were: psychiatric comorbidities, concomitant psychiatric treatments except benzodiazepines, substance abuse, neurological disorders and brain injury.

All subjects provided informed consent to a protocol approved by the local Ethical Committee following the principles of the Declaration of Helsinki.

2.2. Genotyping

DNA was extracted from whole blood by manual extraction, using the "Illustra blood genomicPrep Midi Flow kit" (GE Healthcare, Milan, Italy).

Polymerase chain reaction (PCR) was performed with the following primers: 5′-GGC GTT GCC GCT CTG AAT GC-3′ and 5′-GAG GGA CTG AGC TGG ACA ACC AC-3′.

The PCR reaction was carried out in a 10 µl volume containing 150 ng genomic DNA, 1 µM of each primer, 200 µM of dNTPs (including 7 deaza-dGTP), 1× Hot Master Taq Buffer with Mg++ (Eppendorf), 0.025 U/µl of Hot Master Taq (Eppendorf) and 5% of dimetilsulfoxide DMSO.

The PCR reaction was performed by ABI 9700 PCR thermal-cycler (Applied Biosystems, APPLERA) as follows: after a first step at 94 °C for 2 min, steps of 94 °C for 35 min, 61 °C for 30 min, 70 °C for 65 min for 35 cycles. Then, a final extension step at 70 °C for 8 s was added.

PCR products were separated in 3.5% Seakem agarose gel with ethidium bromide. The bands were visualised by ultraviolet light. The allele including the 44 bp insertion ("L" allele) consists of a fragment of 528 bp, while the allele showing the 44 bp deletion ("S") consists of a fragment of 484 bp.

2.3. Assessment

Psychopathology was assessed by means of the Positive and Negative Syndrome Scale for Schizophrenia (PANSS) (Kay et al., 1987), administered by a trained psychiatrist.

Neuropsychological performances were assessed with computerized Wisconsin Card Sorting Test (WCST), for the evaluation of executive functions, and Continuous Performance Test (CPT), for the evaluation of attention (Stratta et al., 2004), both administered by a trained psychologist in the same session with approximately 30 s pause between WCST and CPT.

Basic clinical and demographic data were collected from clinical reports.

2.4. Data analysis

Analysis of variance (ANOVA) was performed to examine genotype group effects with demographic, clinical and neuropsychological performances as dependent variables and 5-HTTLPR genotype as independent factor. We carried the analysis on main scores of WCST (number of categories, total administered cards, conceptual level responses, perseverative errors, non perseverative errors and failure to maintain set) and CPT (number of hits, missed, responses to distracting stimuli and false alarms). The ANOVA was also used to analyze single effects of gender and treatment and their interaction