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Allelic Variants of the Functional Promoter Polymorphism of the Human Serotonin Transporter Gene is Associated with Auditory Cortical Stimulus Processing

Jürgen Gallinat*[,1], Daniel Senkowski[2], Catrin Wernicke[3], Georg Juckel[4], Isabell Becker[1], Thomas Sander[1], Michael Smolka[1], Ulrich Hegerl[4], Hans Rommelspacher[3], Georg Winterer[1] and Werner M Herrmann[1]

[1]Department of Psychiatry, Free University of Berlin, Berlin, Germany; [2]Department of Clinical Neurobiology, Free University of Berlin, Berlin, Germany; [3]Max-Planck-Institute of Cognitive Neuroscience, Leipzig, Germany; [4]Department of Psychiatry, Ludwig-Maximilians-University, Munich, Germany

The loudness dependence (LD) of the auditory-evoked N1/P2 component has been shown to be related to the central serotonergic neurotransmission. Allelic variants in the promoter region of the 5-hydroxytryptamine transporter (5-HTT) gene were shown to modulate serotonergic activity. It was hypothesized that the three genotypes (l/l, s/l, s/s) differ with respect to LD. Allelic variants of the 5-HTT promoter region and LD at the Cz electrode were determined in 185 healthy subjects prospectively. A significant association was found between LD and genotype (ANOVA: $F = 4.172$, $p = 0.017$). Individuals homozygous for the l allele exhibited a weaker LD compared to heterozygous subjects. The results are consistent with the reported association between 5-HTT genotype and serotonin transport capacity in lymphoblasts, and indicate that auditory stimulus processing is associated with genetic variants of the brain serotonergic system. The LD may serve as endophenotype in human serotonin research.
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INTRODUCTION

A valid indicator of central serotonergic neurotransmission would be useful for various diagnostic and psychopharmacological purposes in psychiatry because peripheral serotonergic measures only partially reflect brain serotonergic function. Since evidence for a modulation of the loudness dependence (LD) of the auditory-evoked N1/P2 component by changes of the central serotonergic activity was reported in humans (von Knorring and Perris, 1981) and animals (Juckel *et al*, 1997) the LD was hypothesized to be such an indicator (Hegerl and Juckel, 1993). The LD denotes the amplitude change of auditory-evoked potentials (AEPs) in response to different stimulus intensities. A strong LD is supposed to indicate a low serotonergic activity and *vice versa*. For example, the LD in behaving cats was found to decrease by application of the 5-HT1a-receptor agonist 8-OH-DPAT and to increase by the 5-HT2-receptor antagonist ketanserin (Juckel *et al*, 1997). Of clinical interest are observations that a strong LD in depressed patients is related to a favorable therapeutical outcome to serotonin agonistic agents (Hegerl and Juckel 1993, Gallinat *et al*, 2000). Furthermore, a strong LD was described in abstinent ecstasy users who are supposed to possess a diminished serotonergic activity (Tuchtenhagen *et al*, 2000), while patients with a serotonin syndrome during SSRI treatment were shown to have a weak LD (Hegerl *et al*, 1998).

However, it is unknown whether LD is modulated by genetic variants of serotonergic neurotransmission. A polymorphic site in the promoter region of the 5-HTT gene comprising a long (l) and a short (s) variant has become a focus in psychiatric research (Lesch and Mossner, 1998). The l/l genotype was described as being associated with higher 5-HT uptake than s/l and s/s in lymphoblasts (Lesch *et al*, 1996) and in nucleus raphe (Heinz *et al*, 2000). Owing to the pivotal role of the 5-HTT in brain 5-HT homeostasis, the SLC6A4 promoter was genotyped in 185 healthy subjects to test if genotypes differ with respect to the LD.

METHODS

Subjects

The study was approved by the ethics committee of the University-Hospital Benjamin-Franklin, Berlin. The subjects gave written informed consent. A total of 185 healthy

*Correspondence: Dr J Gallinat, Department of Psychiatry, Free University of Berlin, Eschenallee 3, 14050 Berlin, Germany, Tel: +49 30 8445 8610, Fax: +49 30 306 85 122, E-mail: juergen.gallinat@charite.de
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unrelated participants (age 39.3 ± 13.4 years, 96 male), all of German origin except five subjects (Austrian, Danish, British), were recruited by newspaper advertisements. A screening was performed by telephone, and a psychiatric interview (M.I.N.I, Sheehan *et al*, 1998) was applied just before LD recording to evaluate the exclusion criterion: axis-I or axis-II disorders, axis-I diagnosis of first-degree relatives, psychotropic drug intake, hearing disorder (whisper test and in doubtful cases 1000-Hz tone audiometry) and any condition that may interfere with the purpose of the study.

AEP-Recording

AEP recording (Synamps-Neuroscan®) was performed with eyes open. Tones (1000 Hz, 40 ms duration, ISI 1800–2200 ms) of five intensities (79, 87.5, 96, 104.5, and 113 dBSPL) were presented binaurally by headphones. At least 30 artefact-free (± 100 μV) sweeps/intensity were averaged. N1 peaks (50–150 ms) and P2 peaks (100–250 ms) were determined semiautomatically at a Cz electrode (linked-mastoids reference). The LD was calculated as a linear regression slope with stimulus intensity as an independent variable and N1/P2 amplitude as a dependent variable (Gallinat *et al*, 2000). Genotyping was performed as described previously (Sander *et al*, 1998).

RESULTS

An ANOVA revealed significant differences of the LD between the three 5-HTT genotype groups ($F(2,182) = 4.172$, $p = 0.017$, $R^2 = 0.044$). *Post hoc* analyses yielded a significant lower LD for the l/l as compared to the s/l genotype ($p = 0.022$), but not for l/l *vs* s/s genotype ($p = 0.15$, Bonferroni, Figure 1). No age differences were found among l/l (40.8 ± 14.2 years), s/l (38.7 ± 12.2 years) and s/s (37.0 ± 13.8 years) genotype groups ($F(2,182) = 1.047$, $p = 0.353$).

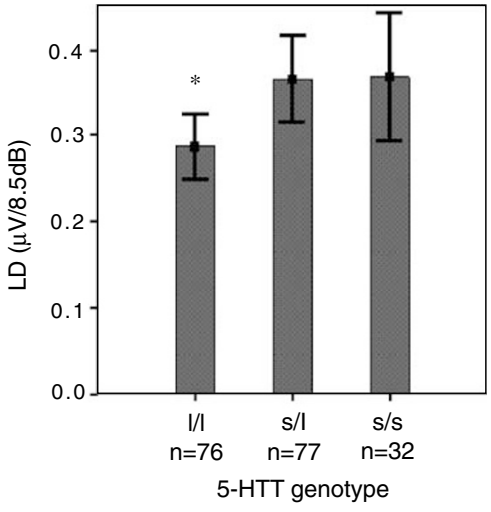

Figure 1 Mean values and standard error of mean of the LD for three genotypes. The l/l homozygous group has a significant weaker LD as compared to the s/l group (*$p = 0.022$).

DISCUSSION

A significantly weaker LD was observed in subjects with l/l genotype as compared to the group with s/l genotype, which may indicate a higher central serotonergic activity in subjects homozygous for the l allele. The 5-HTT genotype explained 4% of LD variance. This finding is important since it confirms and extends a recent SPECT study (Heinz *et al*, 2000) in suggesting that central serotonergic activity is increased in l/l carriers. The present results are also in agreement with the observation that lymphoblasts with one or two s alleles possess a lower 5HT-uptake than cells with the l/l genotype (Lesch *et al*, 1996), although *in vivo* measures showed conflicting results (Willeit *et al*, 2001).

A weak LD was originally interpreted as a consequence of a central mechanism protecting the organism from sensory overload (Buchsbaum, 1976). The later described association of LD with serotonergic neurotransmission (Hegerl and Juckel, 1993) is supposed to be based on the dense serotonergic innervation of the primary auditory cortex—the main N1/P2 generator—especially layer IV (Lewis *et al*, 1986). Since layer IV also receives most of the specific thalamic sensory input, brainstem serotonergic projections are in a position to modulate initial cortical signal processing (Morrison *et al*, 1982) and therefore the LD (Juckel *et al*, 1997). It was reported in behaving cats that the LD is weak during high firing rate of serotonergic neurons in dorsal raphe nucleus and *vice versa* (Juckel *et al*, 1999). It can be speculated that the weak LD of the l/l genotype in the present data is because of a high firing rate of raphe neurons. In line with this, a higher transport capacity of the l/l genotype was suggested to exert a somatodendritic 5-HT1a-receptor-mediated negative feedback with an overall increase of 5-HT neurotransmission (Lesch and Mossner, 1998). Such a mechanism might decrease the LD generated in the auditory cortex and may explain the present results.

Interestingly, recent animal studies indicate a primarily inhibitory effect of 5-HT even on nuclei of the ascending auditory pathway including superior olive (Fitzgerald and Sanes, 1999) and inferior colliculus (Hurley and Pollak, 1999). Furthermore, 5-HT exerts influences on neural development and probably on plasticity and function of the auditory system as well (Fitzgerald and Sanes, 1999). Therefore, it cannot be excluded that 5-HT-genotype-driven developmental alterations of subcortical structures and the ascending auditory pathway underlie the present results.

In summary, LD as a correlate of the auditory cortical stimulus processing may serve as an intermediate phenotype indicating central serotonergic activity as a function of genotype. The LD may be helpful to evaluate the functional significance of other polymorphisms of the serotonin system.

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