Serotonin transporter promoter polymorphism influences topography of inhibitory motor control

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Abstract

The prefrontal cortex participates in motor control and is modulated by serotonergic activity. The serotonin transporter (5-HTT) is a major regulator of serotonergic neurotransmission and may thus influence motor control. The short allele (s) of the 5-HTT linked polymorphic region (5-HTTLPR) is associated with less 5-HTT expression and function than the long variant (l). The neurophysiological parameters termed ‘Go- and NoGo-centroid location’ represent characteristic brain electrical substrates of the execution and inhibition of motor response elicited by the Continuous Performance Test (CPT). In the present study, the impact of the 5-HTTLPR genotype on the centroid locations was investigated in 23 healthy subjects. The NoGo-centroid, but not the Go-centroid, was located significantly more anteriorly in the short allele group (mean electrode location in s/s and s/l, 2.86 ± 0.37) compared to the group with two long alleles (l/l, 3.34 ± 0.49; t = 2.66, p < 0.05). Age, gender, and test performance did not differ between groups. The results indicate that 5-HTTLPR genotype dependent 5-HTT function is associated with the neurophysiologically assessed topography of inhibitory motor control and provides further evidence for a genetic influence on central serotonergic and motor function.

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Introduction

Topographical analysis of event-related potentials (ERPs) elicited during a Continuous Performance Test (CPT), a neuropsychological task requiring the execution (Go-condition) and the inhibition (NoGo-condition) of an anticipated motor response, produces characteristic neurophysiological activation patterns. In a recent ERP study in healthy subjects, we demonstrated that the centre of gravity of the positive brain electrical field during the NoGo-condition was located more anteriorly compared to the Go-condition, a finding which has been addressed as NoGo-anteriorization (NGA; Fallgatter et al., 1997, Fallgatter and Strik, In Press). Based on source location studies with the LORETA method (Pascual-Marqui et al., 1994) an electrical hyperactivity of predominantly right-lateralized prefrontal brain areas was found, particularly during the NoGo-condition (Strik et al., 1998). This result attributes the right-frontal activation, already described, as a global effect during performance of the CPT in metabolic studies with positron emission tomography (PET; Buchsbaum et al., 1990) and near-infrared spectroscopy (NIRS; Fallgatter and Strik, 1997) exclusively to the inhibition of a motor response. Taken together these findings indicate prefrontal brain areas as the topographical brain location of physiological inhibitory motor control.

The activity of the midbrain raphe serotonin (5-HT) system is correlated with the level of behavioural arousal and motor activity (Geyer, 1996). After release by presynaptic neurons the action of 5-HT is primarily terminated by re-uptake via the 5-HT transporter (5-HTT). Thus, the 5-HTT plays a pivotal role in regulating serotonergic neurotransmission in numerous projection fields throughout the brain, including regions critical to the control of motor activity (Hensler et al., 1994). Consistent with this view, the 5-HTT mediates the locomotor action of amphetamine analogues, including 3,4-methylenedioxy-methamphetamine (MDMA) (Bengel et al., 1998; Geyer, 1996). Moreover, frontal 5-HT2A receptor binding was correlated with motor activity in rats with frontal brain lesions (Mayberg et al., 1990). In
another study both, head-weaving and immobility in rats was attributed to an increased serotonergic transmission in the prefrontal cortex (Yamaguchi et al., 1986). These findings indicate that 5-HT is involved in both, execution and inhibition of motor activity in prefrontal brain areas.

Our group previously described a functional length variation polymorphism in the regulatory region of the 5-HTT gene (5-HTT linked polymorphic region, 5-HTTLPR; Heils et al., 1996). This polymorphism has two common forms, a long (l) and a short (s) allele. The s variant is associated with low 5-HTT gene transcription in cell lines and human postmortem brain tissue, resulting in reduced transporter mRNA and protein levels as well as 5-HT uptake, and acts as the dominant allele (Lesch et al., 1996; Litte et al., 1998). Recently, additional rare alleles of the 5-HTTLPR have been described (Lesch et al., 1997; Michaelovsky et al., 1999).

While these findings have encouraged ongoing research exploring possible associations between the 5-HTTLPR variants and personality as well as dimensions of categorically defined neuropsychiatric disorders (for review see Lesch and Mössner, 1998), there has been surprisingly little research investigating allelic variation of 5-HTT expression in electrical or metabolic correlates of motor control. In view of the evidence above, we investigated the impact of the 5-HTTLPR genotype on the Go- and the NoGo-centroid locations in healthy subjects. Our hypotheses was that the genetically driven 5-HT function is associated with the neurophysiological measures of motor control.

Method

Subjects

Twenty-three healthy employees of the hospital (8 physicians, 11 nurses, 4 domestics; 10 female, 13 male) gave informed consent to the electrophysiological assessment and the 5-HTTLPR genotyping. The design of the study is compatible with the recommendations of the Helsinki Committee. The mean age of subjects was $41.0 \pm 8.8$ (range 28–60) yr. All subjects were self-reported right handers and drug free, none had a life-time history of any neurologic or psychiatric disorders.

Continuous Performance Test

The CPT was conducted in an electrically shielded, sound attenuated, and dimly illuminated room. The subjects were seated in a relaxed position on a comfortable chair at a distance of 1.2 m from a monitor. The applied cued CPT version consisted of different letters presented sequentially and in a pseudo-random order with an interstimulus interval of 1650 ms and a presentation time of 200 ms each, in the centre of the computer screen between two vertical fixation lines. Subjects were instructed to press a response button with the index finger of their right hand as fast as possible every time the letter O was directly followed by the letter X. Thus, the letter O was a signal to prepare a motor response (80 primer conditions), whereas X served as target when directly following an O (40 Go-conditions). The other ten letters A, B, C, D, E, F, G, H, J and L were either signals to avoid the prepared motor response when they directly followed an O (40 NoGo-conditions) or meaningless distractors when presented next to any other letter than O (240 distractor conditions). Speed and accuracy were equally emphasized during the explanation of the test. Each subject performed a short training session to ensure correct understanding of the instructions. The reaction times for the responses and the number of omission and commission errors (error rate) were measured.

EEG recording

The EEG was recorded with 21 gold cup electrodes placed according to the International 10/20 System and three additional channels at the outer canthi of both eyes and below the right eye for the registration of eye movements. The presentation of each stimulus was registered in a separate trigger channel with a specific marker for each condition. A 32-channel DC amplifier (Brain-star System) and a data acquisition software (Neuroscan) calibrated with an external 100 µV/10 Hz signal, were used. The hardware filter was set to a bandpass from 0.1–70 Hz, the A/D rate was 256 Hz. Recording references were linked mastoids with compensating resistors of 10 kΩ each. All electrode impedances were below 5 kΩ.

Analysis of EEG data

The procedure of analysis has previously been described in detail (Fallgatter et al., 1997, In Press; Fallgatter and Strik, In Press; Strik et al., 1998). Only EEG epochs with no single amplitude value exceeding 98 µV (artifact criterium) within 500 ms after presentation of a Go- or a NoGo-stimulus were used for averaging of the evoked potentials. Based on the spatially oriented and reference-independent methods proposed by Lehmann (1987), the individual P300 latencies in the Go- and the NoGo-condition were determined based on the peaks of the global field power (GFP) in a P300 time window of 277–434 ms post stimulus. The GFP is a one-number estimator of the electrical field strength in multi-channel recordings and corresponds to the standard deviation of all measured potential values at a given point of time. At
5-HTTLPR genotype influences inhibitory motor control

Figure 1. Grand average maps of the brain electrical field at the P300 peak during the NoGo-condition in the S (left) and the L group (right). Red colour indicates the positive area of the brain electrical field.

Statistical analysis

Based on data from the functional analysis of the 5-HTTLPR effect on 5-HTT gene expression (Lesch et al., 1996) all analyses were performed by dichotomizing the genotypes into two groups: Group S for l/s and s/s and Group L for l/l genotypes. Both neurophysiological parameters were normally distributed. Therefore, two-tailed t tests for independent samples were used to compare the locations of the positive Go- and NoGo-centroids in the anterior–posterior location in these two groups. In an additional exploratory analysis age, gender (x² statistic), error rates and reaction times were contrasted between the two groups.

Results

Distribution of l/l, l/s, and s/s 5-HTTLPR genotypes in the sample was 8 (34.8%), 12 (52.2%), and 3 (13.0%), respectively. The genotype distribution did not show significant departure from the Hardy–Weinberg equilibrium. Subjects in Group S (l/s and s/s) showed significantly more anterior location of the NoGo-centroid (mean ± s.d. electrode location 2.86 ± 0.37) compared to Group L with two long alleles (3.34 ± 0.49; t = 2.66, p < 0.05; Figures 1 and 2). This was not true for the

5-HTTLPR genotype

Genomic DNA isolation and polymerase chain reaction (PCR)-based genotyping for the 5-HTTLPR were performed as previously described in detail (Lesch et al., 1996).
A. J. Fallgatter et al.

Figure 2. Left: planar projection of the electrode array on a head shape as viewed from above; superior corresponds to anterior, left to left side of the head. The electrode locations conform to the International 10/20 System and were registered as indicated. The axes with the coordinate values are displayed next to the head shape. Right: NoGo-centroid location in the long (L, l\textsuperscript{l}) and short allele group (S, l\textsuperscript{s} and s\textsuperscript{s}). Smaller values indicate a more anterior NoGo-centroid location (Figure 1), the boxes reflect the standard errors, and whiskers the standard deviations, respectively.

anterior–posterior location of the Go-centroid (3.53 ± 0.72 vs. 3.94 ± 0.35; \(t = 1.52, \text{ns}\)). The post-hoc power of the statistical analyses at an \(\alpha\)-level of 0.05 was 0.60 for the NoGo- and 0.29 for the Go-centroid locations, the effect sizes were 1.02 and 0.64, respectively (Faul and Erdfelder, 1992). Groups S and L did not differ significantly in age (42.7 ± 10.2 vs. 37.9 ± 4.2 yr; \(t = 1.26, \text{ns}\)), gender (female/male: 6/9 vs. 4/4, \(p = 0.78, \text{ns}\)), and performance in the CPT as expressed by reaction times (483.6 ± 134.7 vs. 515.4 ± 114.3 ms) and error rates (1.73 ± 1.83 vs. 1.25 ± 1.39 errors, \(t = -0.57, \text{ns}\)), respectively.

Discussion

The results of the present study revealed a significantly increased anterior location of the NoGo-centroid in Group S with l/s and s/s 5-HTTLPR genotypes compared to Group L. In contrast, no significant topographical difference was found for the Go-centroids, although the absolute locations were also more anterior in Group S. The findings indicate that 5-HTTLPR-dependent 5-HTT expression is specifically associated with physiological inhibitory motor control but not with executive motor control, localized in prefrontal brain areas. These findings provide further evidence for a genetic influence on central serotonergic function. However, a replication study is needed to confirm this novel link between molecular genetics and neurophysiology.

As the applied CPT version is very easy (presentation time 200 ms, interstimulus interval 1650 ms), almost no errors occurred in healthy subjects. Therefore, between-group differences in motor control are apparent only on a neurophysiological and not on a behavioural level.

To our knowledge, this is the first report on an association of genetically driven activity of the serotonergic system and a neurophysiological paradigm. Recently, the stimulus intensity dependence of the auditory evoked N1/P2 component has been proposed as another indicator of central serotonergic activity (Hegerl et al., 1996; Hegerl and Juckel, 1993). However, the results obtained with this parameter are not directly comparable to the NoGo-centroid location, because intensity dependence of the N1/P2 component reflects amplitude changes dependent on serotonergic functioning in primary and secondary auditory cortical areas.

The 5-HTTLPR, a major regulator of 5-HTT expression and thus of central serotonergic activity, is a useful candidate for studies of complex human behaviour in neuropsychological tasks, because there is consistent evidence of the polymorphism’s functional relevance. Serotonergic raphe neurons diffusely project to a variety of brain regions (e.g. cortex, amygdala, hippocampus, and basal ganglia) and play a role in integrating emotion, cognition, and motor function as well as in food intake, sleep, pain, and sexual activity. The diversity of physiological functions is probably due to the fact that 5-HT acts as a master control neurotransmitter within a highly
complex system of neural communication, mediated by multiple pre- and postsynaptic 5-HT receptors, hence orchestrating the activity and interaction of several other neurotransmitter systems.

This apparent role of 5-HT as a developmental modulator in humans and closely related primate species (Lesch et al., 1997), which is likely to be influenced by the variation in the regulatory region of the 5-HTT gene, could result in enduring effects on arousal and motor activity (Jacobs and Fornal, 1997). Motor symptoms have long been used to characterize the effects of central serotonergic functioning. The prevailing view has been that 5-HT typically inhibits motor output in relation to catecholaminergic systems, but recent findings indicate that the release of presynaptic 5-HT by indirect agonists leads to a profound locomotor activation (Geyer, 1996). The activating effects of indirect agonists, such as MDMA, are dependent upon the release of 5-HT from presynaptic terminals and are mimicked by direct agonists.

The activating effects of indirect agonists, such as MDMA, (‘ecstasy’) in 5-HT transporter-deficient mice. Molecular Pharmacology 53, 649–655.


References


