



Association between a cannabinoid receptor gene (*CNR1*) polymorphism and cannabinoid-induced alterations of the auditory event-related P300 potential

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ABSTRACT

Numerous studies demonstrated a close relationship between cannabis abuse and schizophrenia with similar impairments in cognitive processing, particularly in P300 generation. Recently, an (AAT)*n* triplet repeat polymorphism within the cannabinoid receptor gene *CNR1* has been found to be associated with both schizophrenia and substance dependence, and to modulate the P300 potential. As previously reported, both acute oral Δ^9 -tetrahydrocannabinol (Δ^9 -THC), the main psychoactive constituent of cannabis, and standardized cannabis extract containing Δ^9 -THC and cannabidiol (CBD) revealed a significant reduction of P300 amplitudes in healthy subjects but did not show any differences among each other. The aim of this study was to investigate whether the (AAT)*n* polymorphism differentially modulates the effects of Δ^9 -THC and cannabis extract on P300 generation in 20 healthy volunteers during an auditory choice reaction task. For the >10/>10 genotype, there was a significant decrease of P300 amplitude as well as a significant prolongation of P300 latency under pure Δ^9 -THC but not under cannabis extract. Moreover, we found a significant correlation between the number of AAT repeats and P300 variables for the Δ^9 -THC condition. Our data thus indicate that the *CNR1* gene seems to be involved in the regulation of the P300 wave as a marker of selective attention and working memory. Moreover, it appears that variations within *CNR1* may differentially alter the sensitivity to the acute effects of cannabinoids on P300 generation in healthy subjects.

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The P300 wave is a cognitive event-related brain potential (ERP) component that reflects attentional resource allocation and active working memory [28]. It is most commonly elicited in the context of an auditory 'oddball' paradigm or a choice reaction task in which a sequence of repetitive standard stimuli is interrupted infrequently and unexpectedly by physically deviant ('oddball') stimuli, designated as the 'target'. Subjects are instructed to indicate their perception of each target by a button press. The amplitude of the P300 is typically largest over the medial and parietal scalp locations, and its peak latency may occur between about 300 and 600 ms after stimulus onset. Brain imaging studies in humans have similarly detected multiple sources of P300-like ERP activity, including limbic, hippocampal and cortical areas [26]. Presumably, the neurochemical substrates of the P300 involve various neurotransmitter systems in the brain [8]. Deficient P300 generation in terms of reduced amplitude and/or delayed latency is a robust finding in schizophrenia [3] as well as in substance dependence [16].

Cannabis is the world's most widely used illicit drug. Δ^9 -Tetrahydrocannabinol (Δ^9 -THC) has been identified as the primary psychoactive constituent of the *Cannabis sativa* plant [11]. The activity of Δ^9 -THC is mediated by agonistic effects at central cannabinoid (CB₁) receptors that are distributed with highest densities in the cerebral cortex, basal ganglia, hippocampus, anterior cingulate cortex and cerebellum [15]. Cannabidiol (CBD) is the second most abundant constituent of *C. sativa*. Preclinical evidence has suggested neuroprotective and antipsychotic activity of CBD [24,30,41]. In contrast to Δ^9 -THC, CBD has no psychotogenic properties. CBD is a non-competitive antagonist of the CB₁ receptor, thus reducing the anxiogenic, cognitive and perceptual effects of Δ^9 -THC [27].

CB₁ receptors are encoded by the *CNR1* gene [23]. *CNR1* is located on chromosome 6q14–q15 that is included in the schizophrenia susceptibility locus 6q13–q26 [12]. An (AAT)*n* triplet repeat polymorphism within *CNR1* at the 3'-UTR located 18,086 bp away from the exon 4 translational start site has been identified [40]. This (AAT)*n* polymorphism has been found to be associated with substance dependence [1] as well as with schizophrenia, particularly with the hebephrenic subtype [4,22,38]. Yet, other studies failed

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to replicate any association of (AAT)*n* with schizophrenia or psychotic symptoms of mood disorders, presumably due to limited sample sizes and low statistical power of the comparisons [36,37]. Moreover, Johnson et al. [19] demonstrated a significant association of the (AAT)*n* polymorphism and the P300 potential in substance dependence.

Numerous studies demonstrated a close relationship between cannabis use and the development of schizophrenia [14]. Interestingly, cognitive dysfunction associated with long-term or heavy cannabis use showed strong similarities in many respects to the cognitive impairments that have been observed in patients with schizophrenia [32]. Therefore, a cannabinoid hypothesis of schizophrenia has been established [6]. Accordingly, it is suggested that a dysregulation of the endocannabinoid system may be involved in schizophrenia-related cognitive dysfunction, including deficits in P300 generation.

In a recently published study, our working group investigated the acute effects of Δ^9 -THC and standardized cannabis extract on the auditory P300 potential in healthy subjects [29]. In comparison to placebo, both Δ^9 -THC and cannabis extract containing Δ^9 -THC and CBD revealed significant deficits in P300 amplitudes. Although CBD has been found to reduce several psychotropic effects of Δ^9 -THC, the effects of cannabis extract on P300 did not differ from those of the pure Δ^9 -THC. In combination with the observation of an involvement of the endocannabinoid system in schizophrenia-related cognitive dysfunction, we hypothesized, within the same study sample, that the (AAT)*n* triplet repeat polymorphism in the *CNR1* gene (1) is associated with the P300 potential in healthy human subjects, and (2) differentially modulates the effects of Δ^9 -THC and cannabis extract on P300 generation.

Twenty-seven healthy, right-handed and normally hearing Caucasian subjects were screened and randomised to the study. Two male and five female subjects were excluded from analysis due to technical problems during ERP recording, insufficient quality of the recording or hypersensitivity towards the study medication in terms of panic attack. The remaining 20 subjects (10 male, 10 female, mean age 28.2 ± 3.1 years) finished the study according to the protocol. All subjects had sporadic exposure to cannabis in the past, but according to their statement in the questionnaire, had been completely drug free for at least one month before the onset of the study. They had no addictive or other psychiatric disease as confirmed by a structured psychiatric interview (mini-SCID) and did not take any medication during the study. The study was approved by the Ethics Committee of the Charité University Hospital Berlin. Written informed consent was obtained from all subjects. All experiments were conducted in accordance with the Declaration of Helsinki.

Liquid extract from *C. sativa* (solvent 96% ethanol) and plant-isolated Δ^9 -THC were prepared by the Society of Cancer Research, Arlesheim, Switzerland. The soft-gelatine capsules containing 2.5 mg Δ^9 -THC, cannabis extract with 2.5 mg Δ^9 -THC and 1.35 mg CBD or placebo (a mixture of mono-, di- and triglycerids and glycerol) were produced by Scherer GmbH & Co. KG, Eberbach, Germany.

The study was performed in a prospective, randomized, double-blind, placebo-controlled cross-over design. On three consecutive weeks, each subject was administered four capsules with either Δ^9 -THC (total dose of 10 mg), cannabis extract (total dose of 10 mg Δ^9 -THC and 5.4 mg CBD) or placebo. No alcohol, nicotine or caffeine was allowed the day before the test until the end of the test in order to avoid behavioral and pharmacological interactions with the study medication. All subjects were either non-smokers ($n=15$) or sporadic smokers ($n=5$) with a maximum of 5 cigarettes per day. No nicotine withdrawal was observed nor reported. One hour before each administration, the urine of the subjects was checked for amphetamines and

ecstasy, benzodiazepines, cannabinoids, cocaine, methadone and opiates.

ERP recording was performed 3 h after drug administration and took about 15 min. As previously described in more detail [10], the subjects were presented with a pseudorandomised sequence of 2×30 tone stimuli of different frequencies (1000 and 2000 Hz, 10 ms rise/fall times) within a choice reaction task to elicit auditory evoked ERPs. During the experiment, subjects had to switch off the tones as soon as possible by pressing one of two buttons. The low tones (50%) had to be switched off with the left hand and the high tones (50%) with the right hand. All the stimuli were pure tones with 80 dB SPL. The tones were presented via headphones to both ears at a variable interstimulus interval (ISI: 2.5–7.5 s). The auditory ERPs were recorded by 32 Ag–AgCl electrodes referred to FCz. The electrodes were placed according to the International 10/20 System. Eye movements were monitored using an electrode located 1 cm below the left outer canthus. Impedances were kept below 5 k Ω . Epochs with amplitudes that exceeded $\pm 100 \mu V$ at any electrode were excluded from further averaging. Following artifact rejection and baseline correction, epochs were averaged off-line for each subject and were digitally filtered with a high-pass filter of 0.5 Hz (24 dB/oct) and a low-pass filter of 20 Hz (24 dB/oct). P300 was average referenced and defined as the largest positive deflection occurring during the 250–600-ms latency range after stimulus onset. P300 was determined semi-automatically with a visual control at the midline electrode positions Fz, Cz and Pz.

DNA was extracted from peripheral leukocytes by standard procedures [25]. For fragment analysis of the (AAT)*n* triplet repeat (rs10591494) we used a universal fluorescence labelled, tailed oligonucleotide added to the 5'-part of the sequence specific primer (5'-CATCGCTGATTCGCACAT). Primers were designed with the Primer Express 2.0 Software (Applied Biosystems, Foster City, USA). Three primers were used for PCR: tailed forward primer (CATCGCTGATTCGCACATTAACCTGCCATTAAGGGAA), reverse primer (TCCATGTGTTCTCATTGTCAA) and labelled primer corresponding to the 5'-tail sequence of tailed forward primer. Genotyping was performed on the Beckman Coulter CEQ8000 8-capillary system using 'Fragment Analysis Module' software (Beckman Coulter Inc., Fullerton, USA). Lengths of the repeat units were determined by sequencing homozygous samples.

Statistical calculations were based on nonparametric tests given the relatively small sample size. Values were expressed by means $\pm$ standard deviation. In analogy to Ballon et al. [1], the study sample was split at the median on the number of AAT repeats into two genotype groups ($\leq 10/\leq 10$ or $\leq 10/>10$ ($n=11$) vs. $>10/>10$ ($n=9$) repeats). P300 amplitude and latency for Fz, Cz and Pz under the different medication conditions were compared for the two genotype groups using the Mann–Whitney test. Spearman's correlation coefficients were calculated to determine relationships between the number of AAT repeats and the electrophysiological variables. Statistical significance was taken as $p \leq 0.05$. All statistical analyses were carried out by using the statistical analysis software package SPSS 17.0® (Munich, Germany).

For the (AAT)*n* triplet repeat polymorphism within the *CNR1* gene, a total of 10 alleles were observed corresponding to 7–15 AAT repeats. The same number of alleles has been found in other populations [38].

The P300 amplitudes and latencies by *CNR1* genotype for the three medication conditions are given in Tables 1 and 2. Group comparisons for the *CNR1* genotype revealed a significantly smaller P300 amplitude under the Δ^9 -THC condition in subjects carrying the $>10/>10$ genotype for electrode position Fz ($Z=-2.545$, $p=0.010$), while the differences for Cz and Pz were not significant (Fig. 1a). Moreover, the P300 latency was significantly delayed for the subjects with the $>10/>10$ genotype for Fz ($Z=-2.397$, $p=0.016$) and Cz ($Z=-2.470$, $p=0.012$), both under the Δ^9 -THC condition

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