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Chronic effects of cannabis use on the auditory mismatch negativity

Lisa-Marie Greenwood
University of Wollongong, lmg001@uowmail.edu.au

Samantha J. Broyd
University of Wollongong, sbroyd@uow.edu.au

Rodney Croft
University of Wollongong, rcroft@uow.edu.au

Juanita Todd
University of Newcastle

Patricia T. Michie
University of Newcastle

See next page for additional authors

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Abstract

Background Cannabis use is associated with the development of psychotic symptoms and increased risk for schizophrenia. The mismatch negativity (MMN) is a brain event-related potential marker of change detection thought to index glutamatergic N-methyl-D-aspartate receptor-mediated neurotransmission, which is known to be deficient in schizophrenia. This study examined auditory MMN in otherwise healthy chronic cannabis users compared with nonuser control subjects. Methods Forty-two chronic cannabis users and 44 nonuser healthy control subjects completed a multi-feature MMN paradigm, which included duration, frequency, and intensity deviants (deviants 6%; standards 82%). The MMN was compared between users and control subjects as well as between long- and short-term users and age- and gender-matched control subjects. Associations between MMN, cannabis use measures, and symptoms were examined. Results The MMN amplitude was significantly reduced to frequency but not duration or intensity deviants in overall cannabis users relative to control subjects. Frequency MMN was similarly attenuated in short- and long-term users relative to control subjects. Long-term users also exhibited reduced duration MMN relative to control subjects and short-term users and this was correlated with increased duration of exposure to cannabis and increased psychotic-like experiences during intoxication. In short-term users, a younger age of onset of regular cannabis use and greater frequency of use were associated with greater psychotic-like experiences and symptomatic distress. Conclusions These results suggest impaired sensory memory that might reflect N-methyl-D-aspartate receptor dysfunction in chronic cannabis users. The pattern of MMN alterations in cannabis users differed from that typically observed in patients with schizophrenia, indicating overlapping but distinct underlying pathology.

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Authors

Lisa-Marie Greenwood, Samantha J. Broyd, Rodney Croft, Juanita Todd, Patricia T. Michie, Stuart Johnstone, Robin Murray, and Nadia Solowij

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Archival Report

Chronic effects of cannabis use on the auditory mismatch negativity

Lisa-marie Greenwood, Samantha J. Broyd, Rodney Croft, Juanita Todd, Patricia T. Michie, Stuart Johnstone, Robin Murray, and Nadia Solowij

From the School of Psychology and Ψ-P3: Centre for Psychophysics, Psychophysiology and Psychopharmacology, University of Wollongong, Wollongong NSW Australia (LG, SB, RC, SJ, NS); School of Psychology and Priority Research Centre for Translational Neuroscience and Mental Health, University of Newcastle, Newcastle NSW Australia (JT, PM); Institute of Psychiatry, Kings College, London UK (RM); Schizophrenia Research Institute, Sydney NSW Australia (JT, PM, NS).

Address correspondence to Nadia Solowij, PhD, Associate Professor, School of Psychology, University of Wollongong NSW Australia; E-mail: nadia@uow.edu.au; Tel. +61 2 4221 3732; Fax. +61 2 4221 4163.

Keywords: cannabis, schizophrenia, mismatch negativity, MMN, sensory memory, event-related potentials

Abstract

Background: Cannabis use is associated with the development of psychotic symptoms and increased risk for schizophrenia. The mismatch negativity (MMN) is a brain event-related potential marker of change detection thought to index glutamatergic N-methyl-D-aspartate (NMDA) receptor mediated neurotransmission, which is known to be deficient in schizophrenia. This study examined auditory MMN in otherwise healthy chronic cannabis users compared with nonuser controls.

Methods: Forty-two chronic cannabis users and 44 nonuser healthy controls completed a multi-feature MMN paradigm, which included duration, frequency and intensity deviants (deviants 6%; standards 82%). MMN was compared between users and controls, as well as between long- and short-term users and age- and gender-matched controls. Associations between MMN, cannabis use measures and symptoms were examined.

Results: MMN amplitude was significantly reduced to frequency but not duration or intensity deviants in overall cannabis users relative to controls. Frequency MMN was similarly attenuated in short- and long-term users relative to controls. Long-term users also exhibited reduced duration MMN relative to controls and short-term users and this was correlated with increased duration of exposure to cannabis and increased psychotic-like experiences during intoxication. In short-term users, a younger age of onset of regular cannabis use and greater frequency of use were associated with greater psychotic-like experiences and symptomatic distress.

Conclusions: These results suggest impaired sensory memory that may reflect NMDA receptor dysfunction in chronic cannabis users. The pattern of MMN alterations in cannabis users differed from that typically observed in patients with schizophrenia, indicating overlapping but distinct underlying pathology.

Introduction

Cannabis use is associated with adverse health outcomes and mental disorders (1-4). In combination with a range of other risk factors, including genetic vulnerability (5-7), cannabis use is considered by some to be a component cause of schizophrenia (8-10), with evidence for a dose-response relationship increasing the risk of developing psychosis by 50-200% (5, 11-12), although alternative explanations for the association have been proposed (13). While only a minority of users develop psychosis (10), cannabis has been estimated to be responsible for 14% of psychotic cases (11). Δ^9-Tetrahydrocannabinol (THC), the primary psychoactive constituent of cannabis, can induce psychotic-like symptoms and impair cognition (8), due in part to its action as a partial agonist at central cannabinoid receptors (CB1R) (14) and downstream effects on neurotransmitters such as glutamate and dopamine (15). CB1Rs are among the most abundant receptors in the brain and occur in high density in regions involved in cognition, particularly learning and memory (e.g. hippocampus, prefrontal cortex, anterior cingulate, basal ganglia, cerebellum) (8, 16-17). Chronic exposure to cannabis has been shown to impair endocannabinoid (eCB) regulation of synaptic plasticity via down-regulation of N-methyl-D-aspartate receptors (NMDAR) and reduced glutamatergic and other neurotransmission (18-21). Glutamatergic NMDAR dysfunction is also implicated in schizophrenia (22). Given the propensity for long-term cannabis exposure to induce cognitive impairment, psychotic-like symptoms and functional and structural brain changes that resemble schizophrenia (23-26), further investigation of glutamatergic system functionality in chronic users may inform mechanisms by which cannabis might lead to such phenotypes.

The mismatch negativity (MMN) is a brain event-related potential (ERP) marker of change detection that is robustly reduced in amplitude in schizophrenia (27-28) and thought to be sensitive to NMDAR dysfunction (29): MMN is dose-dependently reduced by the NMDAR antagonist ketamine (30-33) (ketamine-induced NMDAR hypofunction being a well-established model of schizophrenia (34)), while remaining unaffected by monoamine augmentation or attenuation (31, 35). Smaller MMN in healthy volunteers has been shown to predict vulnerability to ketamine-induced psychotic symptoms (32). MMN is elicited by occasional sound deviants in a background of

repetitive sound stimuli (36), requiring current sensory input to be compared to a representation or 'model' of acoustic regularities in memory. MMN elicited to violations of this regularity is a reflection of the dynamic updating of such models, implicating a role of synaptic plasticity in MMN generation (37). Smaller MMN amplitudes are thought to reflect decreased sensory memory formation and abnormal perception or attention, and in schizophrenia patients, are associated with impaired daily functioning and cognitive performance (38). There is speculation, however, that alterations in MMN to different types of deviants are not equivalent indices of underlying pathophysiology. For example, reduced MMN to duration deviants is a robust finding in patients at early stages of schizophrenia, is impaired in the prodromal phase of the illness and in those at risk of developing psychosis (39-41), and is associated with poorer premorbid function (42). Evidence is mixed on whether duration MMN is also reduced in patients in the later stages of the illness (42, 43). In contrast, attenuated MMN to frequency deviants is not reduced in the prodrome (40) and has been associated primarily with chronic schizophrenia illness (43-46). Reduced frequency MMN in schizophrenia patients has been associated with progressive grey-matter loss in left Heschl's gyrus (47-48), and reduced duration MMN with loss in right Heschl's gyrus (47). In summary, reduced MMN amplitude is a robust phenotype in schizophrenia, with a mean effect size of 0.99 (28), but tonal properties may have differential sensitivity to associated neurodevelopmental and neurodegenerative pathology (42).

Given the similarity of phenotypes reported in chronic cannabis users and in schizophrenia, attention has turned to investigating MMN in cannabis users. Roser et al. (49) did not find a difference between cannabis users and controls overall in duration or frequency MMN, but long-term and heavy users showed reduced frequency MMN amplitude compared to short-term and lighter users, and smaller MMN was associated with longer duration cannabis use (49). The same researchers reported enhanced frequency MMN amplitudes after the acute administration of cannabis extract containing THC and cannabidiol (CBD) which they argued reflected the antipsychotic properties of CBD (50-51), and a subsequent analysis suggested that genotype may interact with cannabis in modulating MMN amplitude (52). Another study reported attenuated frequency MMN in chronic cannabis users compared to nonuser controls, while no differences were

observed between chronic cannabis users with and without schizophrenia (53). Interestingly, although both Rentzsch et al. (53) and Roser et al. (49) included a duration deviant, group differences in each of these studies were evident for frequency MMN only. More recently, Roser and colleagues (54) showed that a CB1R antagonist co-administered with ketamine reduced duration MMN more than frequency MMN. Further, Pesa and colleagues reported an altered pattern of duration MMN outcomes in first-episode psychosis patients who used cannabis, relative to patient nonusers (55), with quantity and frequency of recent cannabis use correlating with duration MMN. Thus, there is mixed evidence regarding the effects of cannabinoid exposure on frequency and duration MMN and the extent to which the pattern of effects in chronic users resembles that observed in schizophrenia.

The primary aim of the current study was to further investigate MMN amplitude reduction in chronic cannabis users relative to age- and gender-matched healthy nonuser controls in a multi-feature paradigm with duration, frequency and intensity deviants, and to examine the effect on MMN of duration of regular cannabis use, as well as other cannabis-use parameters, and their association with psychotic-like symptom measures.

Methods and Materials

Participants

Forty-two regular long-term cannabis users (defined as *minimum* fortnightly use for *at least* 2 years) and 44 controls were recruited via newspaper advertisements. Controls had not used cannabis more than 20 times, and not within the past 12 months. Exclusion criteria are described in Supplement 1. Three cannabis users were further excluded for having IQ scores less than 80 and two controls were excluded for unusable EEG data, leaving 39 users and 42 healthy age- (±3 years) and gender-matched controls.

Demographics and substance use history (Table 1) were obtained through structured interview and the Alcohol Use Disorder Identification Test (56). The vocabulary and matrices

subscales of the Wechsler Abbreviated Scale of Intelligence (57) estimated full scale IQ. Measures of psychosis-proneness were obtained using the Community Assessment of Psychic Experiences (CAPE, 58) and Schizotypal Personality Questionnaire (SPQ, 59) and participants also completed the Beck Depression Inventory (BDI, 60) and the Spielberger State-Trait Anxiety Inventory (STAI, 61). The Kessler Psychological Distress Scale (K10, 62) screened for current potential psychiatric conditions. Cannabis users completed the Marijuana Withdrawal Checklist (63) and the Cannabis Experiences Questionnaire (CEQ, 64) to measure symptoms experienced while intoxicated. Participants were asked not to use cannabis, alcohol or any illicit substance for at least 12 hours prior to testing. Urinalysis corroborated self-reported substance use and quantified cannabinoid metabolites (creatinine normalised). Participants provided written informed consent and were reimbursed for their participation. Ethics approval was obtained from the University of Wollongong and Illawarra Shoalhaven Local Health District Health and Medical Human Research Ethics Committee.

Stimuli and task parameters

Participants were presented three 7-minute sequences of 850 tones (SOA=500ms), binaurally through headphones (Sennheiser HD215). Standard tones (82%) were presented at 1000Hz, 80dB SPL and 50ms duration (5ms rise/fall time). Three types of deviant tones (6% each), a duration (100ms), frequency (1200Hz) and intensity deviant (90dB SPL), were presented pseudorandomly in each sequence, with at least two standard tones presented between deviants. Participants were seated in a sound-attenuated booth and watched a silent film, ignoring the tones.

EEG recording and data analysis

EEG data were recorded and processed in a standard manner as described in Supplement 1. Data were averaged to standard tones (excluding the stimulus immediately following the deviant), and each of the deviant types separately. MMN for each deviant type was calculated by subtracting the standard from the deviant waveform, and then lowpass filtered at 20Hz (24dB, zero-phase shift) (65). MMN peak amplitude in each condition was identified as the most negative peak 130-230ms post-stimulus for the duration, and 100-200ms for frequency and intensity conditions, with the

constraint that MMN data referenced to the nose had to exhibit frontal negative topography with polarity reversal at the mastoids. MMN analyses were restricted to Fz (66).

Statistical Analysis

Group comparisons of demographics, clinical measures and psychosis-proneness were conducted using independent samples t-tests (Mann-Whitney U tests for skewed data). Group comparisons of MMN peak amplitudes at Fz were conducted using repeated measures analysis of variance (rmANOVA), with two between-subjects factors, cannabis use group (users vs. controls) and matched duration group (short-term users and matched controls vs. long-term users and matched controls), and a within-subjects condition factor including three levels of deviant (duration, frequency and intensity). The matched duration groups were formed by dividing the users into two groups using a median split for duration of regular cannabis use (short-term < 10 years, n=20; long-term ≥ 10 years, n=19), and then creating two independent subsets of nonuser controls (n=21 each) matched on age and gender to the short- and long-term user groups. Outliers were excluded as reported in Supplement 1.

Spearman's correlations examined associations between MMN amplitude and measures of cannabis use (age started regular use, duration of regular use, frequency and quantity of use, urinary cannabinoid metabolites, self-reported hours since last use, withdrawal scores) and psychosis-proneness.

Results

Demographics, substance use and psychosis-proneness

Cannabis users had used regularly for a median 9.63 years (range 2.3–40.3) and were currently using approximately 4 joints/day on a median 27 days/month (range 3–30; Table 1). Cannabis users and controls did not differ in age, gender or handedness, but users had a lower IQ, fewer years of education, higher SPQ total and CAPE total symptom frequency (but not distress) scores, higher K10, BDI and STAI State and Trait scores, smoked more cigarettes per day, and

consumed more alcohol more frequently than controls (Table 1). As none of these variables correlated with MMN amplitude in any condition they were not included as covariates in analyses. Short-term cannabis users differed from long-term users only in age and duration of regular and daily cannabis use (Table 1). In controls, age did not correlate with duration or frequency MMN amplitude (Spearman's *rho*=.12, *p*=.48; *rho*=.26, *p*=.10, respectively), but correlated with intensity MMN (*rho*=.37, *p*=.02). In cannabis users, age correlated with duration MMN (*rho*=.38, *p*=.02), but not with frequency (*p*=.72) or intensity MMN (*p*=.36). Since age and duration of use were highly correlated (p<.0001), and age did not interact with group in the comparison of short- and long-term users for any condition, age was not included as a covariate in any analysis other than examining the main effect of matched duration groups (since these likely reflected a combination of duration of cannabis use and age). Differences between short- or long-term users and their matched controls (Table 1) and associations between cannabis use and psychosis-proneness measures are discussed in Supplement 1.

MMN analysis

Mean amplitudes for standard ERPs over the latency windows used for MMN peak identification did not differ significantly between cannabis users and controls (Figure S1 in Supplement 1). Figure 1 shows MMN waveforms and Table 2 group means and standard deviations for MMN amplitudes to each deviant type. The rmANOVA results indicated a condition by cannabis use group interaction [$F(2,144)=3.55$, $p=.03$] and a three way interaction between condition, cannabis use group and matched duration group [$F(2,144)=6.25$, $p=.002$]. There was also a main effect of condition [$F(2,144)=84.6$, $p<.001$], no interaction between condition and matched duration group ($p>.15$) and no main effect of cannabis use group ($p=.24$), but there was a main effect of matched duration group [$F(1,72)=5.62$, $p=.02$]. The inclusion of age as a covariate eliminated the latter ($p=.74$) but otherwise did not change the pattern of significant results [condition by cannabis use group, $F(2,142)=3.80$, $p=.025$; condition by cannabis use group by matched duration group, $F(2,142)=5.85$, $p=.004$]. The significant interactions (Figure 2) were decomposed through univariate ANOVAs for each deviant type by comparing cannabis users and controls overall, and then short-

and long-term cannabis user groups with each other and their respective matched control subgroups, followed by further post hoc tests to determine whether the differences between MMN deviant types differed between groups.

MMN in regular cannabis users and controls

Frequency MMN was reduced in cannabis users overall [$F(1,76)=12.90$, $p=.001$] and remained significant after controlling for multiple comparisons, while no differences were observed for duration [$F(1,79)=.35$, $p=.56$] or intensity MMN [$F(1,76)=.02$, $p=.88$]. Post hoc tests confirmed that the group difference was largest in the frequency deviant condition (Supplement 1).

However, duration MMN amplitude correlated positively with the duration of daily cannabis use (*rho*=.54, $p=.003$) (also duration since first tried, *rho*=.36, $p=.03$, and overall duration of regular use trend, *rho*=.30, $p=.07$) (Figure 3), indicating reduced duration MMN with longer periods of use. The association with duration of daily use remained significant after controlling for age (partial $r=.38$, $p=.046$) whereas there was no association with age after controlling for duration of daily use ($p=.45$). No other cannabis use, psychosis-proneness or other clinical measure correlated significantly with MMN for any deviant condition.

MMN in short- and long-term user subgroups

Frequency MMN in short- and long-term users did not significantly differ ($p>.39$), however both groups had smaller frequency MMN than their matched controls [short-term users, $F(1,39)=7.71$, $p=.008$; long-term users, $F(1,35)=7.97$, $p=.008$]. Duration MMN was smaller in long- relative to short-term users [$F(1,37)=12.17$, $p=.001$], consistent with the correlation between duration MMN and duration of use in the full sample of users. Long-term users also had reduced duration MMN compared to their matched controls [$F(1,38)=4.24$, $p=.046$], whereas short-term users did not differ from their matched controls ($p>.65$). No group differences were identified for intensity MMN (long vs. short: $p>.10$; long and short vs. respective controls: $p>.76$). Post hoc tests confirmed that the largest group difference between long- and short-term users and between long-

term users and their matched controls was in the duration condition, and that between short-term users and their matched controls in the frequency condition (Supplement 1).

No psychosis-proneness, other clinical or cannabis use measure correlated with MMN in any condition in short-term users. In long-term users, duration MMN was positively correlated with the duration of daily use (*rho*=.61, *p*=.017) (Figure 3), remaining significant after controlling for age (partial *r*=.60, *p*=.023), whereas the association with age was not significant after controlling for duration of daily cannabis use (*p*=.92). CEQ psychosis-like symptoms were positively correlated with duration (*rho*=.49, *p*=.04) and frequency MMN (*rho*=.51, *p*=.03) (Figure 4) in long-term users, indicating an association between reduced MMN amplitude and greater positive psychosis-like symptoms while intoxicated. No other associations with clinical measures were identified.

Discussion

We report the first study to employ a multi-feature paradigm to examine MMN to duration, frequency and intensity deviants in regular cannabis users and age-matched controls. Consistent with previous studies (53, 49), we found reduced frequency MMN in the overall sample of users relative to controls, whereas duration MMN did not differ between groups and in a novel result, neither did intensity MMN. Long- and short-term users showed reduced frequency MMN relative to their matched control groups and in addition, long-term users showed reduced duration MMN relative to both short-term users and age-matched controls, an effect that was larger than their reduced frequency MMN relative to controls.

While frequency MMN was unrelated to cannabis use measures, duration MMN decreased with years of daily exposure to cannabis in the full sample of users and in the long-term user group. A relationship between intensity MMN and duration of daily cannabis use only emerged after controlling for alcohol use (Supplement 1). No MMN measures showed significant correlations with psychosis-proneness measures, but both frequency and duration MMN reductions in long-term users were related to increased positive symptoms while intoxicated (as assessed by the CEQ).

Reduced MMN is a robust phenotype in schizophrenia and the current data add to a growing literature demonstrating reduced MMN in cannabis users, particularly long-term users. However, the pattern of effects of chronic cannabis use on MMN amplitude to different deviant types contrasts with that observed in schizophrenia. Although direct comparisons are difficult, the paradigm utilised here is very similar to that reported by Todd et al. (43) where reduced frequency MMN in schizophrenia patients was associated with longer durations of illness, while attenuated duration and intensity MMN were associated with earlier stages of illness (see also 28). In the current study, long- and short-term cannabis users showed a similar degree of frequency MMN reduction, but duration MMN showed greater attenuation with longer exposure to cannabis. Following prolonged exposure (near daily for >20 years on average), long-term users may have developed schizophrenia-like changes in the brain (67) in terms of structural and neurochemical changes similar to those associated with conversion to psychosis (40, 68). This is consistent with findings of attenuated duration MMN in individuals at high risk of developing psychosis (40) and patients with first-episode psychosis (39, 55). Despite their protracted heavy use, the long-term users have not developed schizophrenia, possibly due to a range of protective factors, and are unlikely to given their age. Intact duration MMN in short-term users might also reflect resilience before a threshold of exposure to cannabis is reached, beyond which detrimental effects on duration MMN occur as in long-term users, particularly with daily use. Of note, however, short-term users exhibited higher psychosis-proneness scores than matched controls, were more symptomatic on psychosis-proneness measures than long-term users and their symptoms more closely associated with cannabis use measures, and when intoxicated short-term users experienced greater paranoia and reduced euphoria compared to long-term users (Table 1 and Supplement 1). An alternative explanation for the MMN patterns observed here is that duration MMN may be a less sensitive index of impairment associated with cannabis use than frequency MMN, until use becomes quite protracted, reflecting potentially different neural processes and/or toxicity underlying reduced MMN to each deviant type.

In schizophrenia, reduced frequency (and to a lesser extent duration) MMN amplitude is correlated with grey matter volume loss in auditory and frontal regions (48). Increased grey matter loss over time, as well as cortical thinning, has been reported in first-episode patients who used cannabis compared to those who didn't (69-70) and abnormal gyrification and cortical thinning reflecting premature aging have been reported in otherwise healthy young cannabis users of similar characteristics to those in our study (71). The abnormal gyrification (less concave sulci in left frontal and bilateral temporal lobes and thinner sulci in right frontal lobe than in controls) was not associated with age, age of onset, duration or cumulative exposure to cannabis. On the other hand, gyrification and other morphological abnormalities are age- and disease-related and progressive in schizophrenia (72-75). While no longitudinal studies have examined whether brain morphological alterations are progressive in cannabis users, the lack of a duration of exposure effect suggests that abnormal gyrification in auditory cortex may develop early in heavy cannabis users and not progress, while other brain structures and connectivity are more impacted by cumulative exposure to cannabis (76, 26). This could explain attenuated frequency MMN evident in both short- and long-term users in this study, given that sound frequency is encoded by tonotopic maps across superior temporal lobe surface (77), whereas duration MMN may be more sensitive to damage to multiple brain regions and connectivity given the distributed nature of sound duration encoding across networks (78-81). Indeed, the size of MMN depends on frontotemporal connectivity and MMN-like activity has been demonstrated in the hippocampus (81, 82). We have reported both hippocampal volumetric reduction (26) and altered structural (83) and functional (84) connectivity in long-term heavy cannabis users, which may contribute to the aberrant MMN patterns observed here, particularly for duration MMN.

Another potential contributor to the observed pattern of MMN results is a direct effect of cannabis on the auditory system. Auditory discrimination performance has been shown to be sensitive to disruption by THC in rats (85). Cannabinoid receptors are important in the control of hearing (86) and influence perceptual and mnemonic aspects of auditory experience (87). The eCB system plays an important role in developing auditory circuits (88), which are disrupted by exposure

to cannabinoids (89). Further, this system is thought to modulate the balance of excitation and inhibition in auditory circuits (90), upon which experience-dependent alterations in neuronal firing patterns depend (91). The eCB system is also involved in plasticity in the dorsal cochlear nucleus which is critical for frequency processing (92) and in plasticity, which may be linked with the development of a memory trace for certain stimulus profiles (93). Prolonged exposure to cannabis may disrupt eCB-mediated plasticity and impair the development of an effective memory trace for trains of auditory stimuli. Future research should elucidate the effects of chronic cannabis exposure on the development of a 'model' of acoustic regularities represented by standard stimuli (e.g. see 94). Endocannabinoids are also, in concert with NMDAR, critically involved in coincidence detection (20) which underlies the tuning of synaptic integration that is important for distributed computations (95), such as those involving duration discrimination. Exogenous cannabinoids disrupt the temporal and spatial precision and cohesion of eCB signalling critical for synapse formation (96). Imprecision in encoding sound properties through neural representations in the auditory system and resultant discrimination impairment could contribute to reduced MMN amplitude, but cannot entirely account for the pattern of MMN reduction in schizophrenia (94).

MMN is thought to be dependent on the activity of NMDARs fundamental to glutamatergic transmission, likely in concert with dopamine and other neurotransmitters and neuromodulators, including eCBs. Excessive stimulation and downregulation of CB1Rs (97) by chronic exposure to cannabis disrupts the regulatory role of eCBs on glutamatergic homeostasis and transmission (98, 21, 99-100). Prolonged exposure to cannabis would therefore give rise to aberrant striatal dopaminergic function, as described in individuals at high risk for psychosis (101) in whom reduced duration MMN was found to be related to lower thalamic glutamate plus glutamine levels (102). This suggests that abnormal glutamatergic functioning is present early in the at-risk mental state, but since both duration and frequency MMN index impaired NMDAR function (33), this does not serve to elucidate the differential patterns of duration versus frequency MMN observed in people with schizophrenia or in the cannabis users of this study.

A limitation in the current study is the relatively brief period of abstinence from cannabis at testing (median 14.5 hours). Although MMN was not correlated with hours since last cannabis use, or with withdrawal symptoms, examining the MMN response after longer periods of abstinence may better inform residual, long-lasting or neurotoxic effects of cannabis on MMN. It is possible that a third variable, such as premorbid or global functioning, or indeed genotype, may mediate or moderate the association between cannabis and MMN alterations. While an association between MMN and educational achievement as a measure of premorbid function has been reported (42), we found no association between years of education in cannabis users or controls in this study, and while we did not measure global functioning, evidence for an association with MMN is mixed (42, 103). Finally, as a cross-sectional study it is not possible to determine whether smaller frequency MMN may have existed prior to the onset of cannabis use and reflect a vulnerability to use.

In summary, the current findings suggest that regular exposure to cannabis over a long period is associated with smaller MMN to both frequency and duration deviants that may reflect impairment in either establishing a “model” of acoustic regularities in memory or dynamic updating of the model that is required when a deviant violates the regularity. The observed pattern of results in chronic cannabis users contrasts with the pattern observed in patients with schizophrenia. The results nevertheless implicate altered NMDAR-mediated neural transmission, postulated to result from chronic exposure to cannabis disrupting the eCB system’s role in regulating glutamatergic signalling. Future work should be directed at understanding these mechanisms more precisely by examining acute effects of cannabinoids such as THC and CBD on MMN as a marker of NMDAR function, and establishing the potential genetic modulation of cannabinoid effects on this system to inform vulnerability to psychosis when cannabis is used.

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Table 1. Demographic data, substance use measures and symptoms in cannabis users and healthy nonuser controls.

	Cannabis			Control		
	All (n=39)	Short-term (n=20)	Long-term (n=19)	All (n=42)	Short-term (n=21)	Long-term (n=21)
Demographics						
Handedness (left/right)	2/37	1/19	1/18	2/40	0/21	2/19
Gender (male/female)	25/14	15/5	10/9	25/17	12/9	13/8
Age (years)	26.4 (18.3-54.2)	20.9 (18.3-27.8)	39.9 (26.4-54.2)	27.2 (18.1-52.6)	21.0 (18.1-27.2)	36.1 (27.2-52.6)
Education (years)	12.5 (10-17)**	13.0 (10-17)*	12.0 (10-17)*	13.8 (11-20)	13.7 (12.5-17)	14.0 (11-20)
IQ	107 (86-135)*	104 (86-135)*	108 (94-125)	113.5 (89-133)	113 (94-133)	115 (89-133)
Alcohol Use						
Frequency (days/month)	3 (0-30)*	2.5 (0-10)	6 (0-30)*	2.5 (0-20)	2.5 (0-10)	2.5 (0-20)
Quantity (standard drinks/month)	19 (0-252)*	17.5 (0-195)	22.5 (0-252)*	7.5 (0-70)	9 (0-70)	6 (0-28)
Tobacco (cigarettes/day)	2 (0-35)**	2 (0-12.5)**	6.5 (0-35)*	0 (0-12)	0 (0-4)	0 (0-12)
Psychosis-Proneness						
CAPE Total Frequency	63.5 (42-92)*	64 (42-92)*	61 (50-84)	58 (43-89)	59 (43-86)	56 (43-89)
CAPE Total Distress	26 (0-79)	27 (0-79)	25 (9-53)	21.5 (1-95)	22 (3-61)	21 (1-95)
SPQ Total	21.5 (0-44)*	27 (1-42)*	17.5 (0-44)	11 (0-39)	10 (0-29)	14 (2-39)
CEQ Euphoric	41 (25-72)	44 (26-72)*	39 (25-56)			
CEQ Paranoid	36 (25-76)	44.5 (25-76)*	33 (25-56)			
CEQ After Effects	19.5 (0-50)	21.5 (0-50)	19 (13-33)			
CEQ Amotivational	14 (0-34)	13.5 (0-34)	14 (8-24)			
CEQ Psychosis-like	6 (0-16)	7 (0-16)	6 (4-9)			
Clinical measures						
Kessler Psychological Distress Scale (K10)	15 (11-34)*	18 (11-34)*	15 (11-28)	14 (10-22)	13.5 (11-22)	14 (10-19)
Beck Depression Inventory (BDI)	6 (0-27)*	6 (0-27)*	6 (0-22)*	2 (0-19)	1 (0-13)	2 (0-19)
State Anxiety Index (STAI-I)	31 (20-51)*	30 (20-48)*	33 (20-51)	26.5 (20-45)	26 (20-45)	28 (20-43)
Trait Anxiety Index (STAI-II)	36 (20-65)*	36 (20-65)	35 (23-47)	30 (20-61)	29 (20-61)	31 (20-50)
Cannabis Use						
Hours since last use	14.5 (13-420)	14.0 (13-240)	16.0 (13-420)			
Frequency (days/month)	27 (3-30)	25 (5-30)	30 (3-30)			
Quantity (cones/month)[d]	338 (8-3150)	368 (10-3150)	250 (8-1931)			
Age of first use (years)	14.5 (8-19)	14.3 (10-16)	15.0 (8-19)			
Age started regular use (years)	16.0 (10.0-25.0)	16.0 (10.0-19.5)	16.0 (12.5-25)			
Duration of regular use (years)	9.6 (2.3-40.3)	5.0 (2.3-9.6)**	26.1 (10.4-40.3)			
Duration of daily use (months)	60 (0-360)	29 (0-88)*	102 (0-360)			
Urinary cannabinoid metabolite (THC- COOH) (ng/ml)	628 (0-9351)	618 (0-3658)	716 (0-9351)			

Comparisons were between cannabis groups and matched controls [Cannabis (All) vs Controls (All); Short-term Users vs Short-term Matched Controls; Long-term Users vs Long-term Matched Controls]. Cannabis use measures were compared between short-term users and long-term users.
*p < .05 **p < .001
Data reported as median (range).
[a]Community Assessment of Psychic Experiences (CAPE); [b]Schizotypal Personality Questionnaire (SPQ); [c]Cannabis Experiences Questionnaire (CEQ); [d]Cones used in waterpipe; 3 cones are equivalent to one standard sized joint.

Table 2. MMN Peak Amplitude at Fz.

	Duration MMN Amplitude (μV)	Frequency MMN Amplitude (μV)	Intensity MMN Amplitude (μV)
Cannabis (All)	-5.83 (2.6)	-2.81 (1.1)*	-3.33 (1.8)
Short-term Users	-7.03 (2.8)	-2.95 (1.4)*	-3.79 (2.1)
Long-term Users	-4.56 (1.3)*	-2.64 (0.6)*	-2.86 (1.3)
Controls (All)	-6.20 (2.7)	-4.04 (1.8)	-3.29 (2.0)
Short-term Matched	-6.58 (3.6)	-4.64 (2.4)	-3.59 (1.9)
Long-term Matched	-5.81 (2.3)	-3.44 (1.3)	-3.01 (2.0)

Comparisons between cannabis groups and matched controls [Cannabis (All) vs Controls (All); Short-term Users vs Short-term Matched Controls; Long-term Users vs Long-term Matched Controls] on duration, frequency and intensity mismatch negativity (MMN) amplitude. Standard deviation in parentheses.*$p < .05$

Figure Legends

Figure 1. Mismatch negativity (MMN) waveforms at Fz displayed in each condition for the cannabis user and matched nonuser control groups.

Figure 2. Mean mismatch negativity (MMN) peak amplitude at Fz for the short- and long-term user group and their respective matched control groups. Vertical bars represent the standard error of the mean.

Figure 3. Duration mismatch negativity (MMN) amplitude as a function of duration of regular and daily cannabis use in the overall sample of cannabis users, and as a function of daily cannabis use in the long-term group.

Figure 4. Associations between psychosis-like experiences during cannabis intoxication reported on the Cannabis Experiences Questionnaire (CEQ) and duration and frequency mismatch negativity (MMN) amplitude at Fz in the long-term user group.

Figure 1.

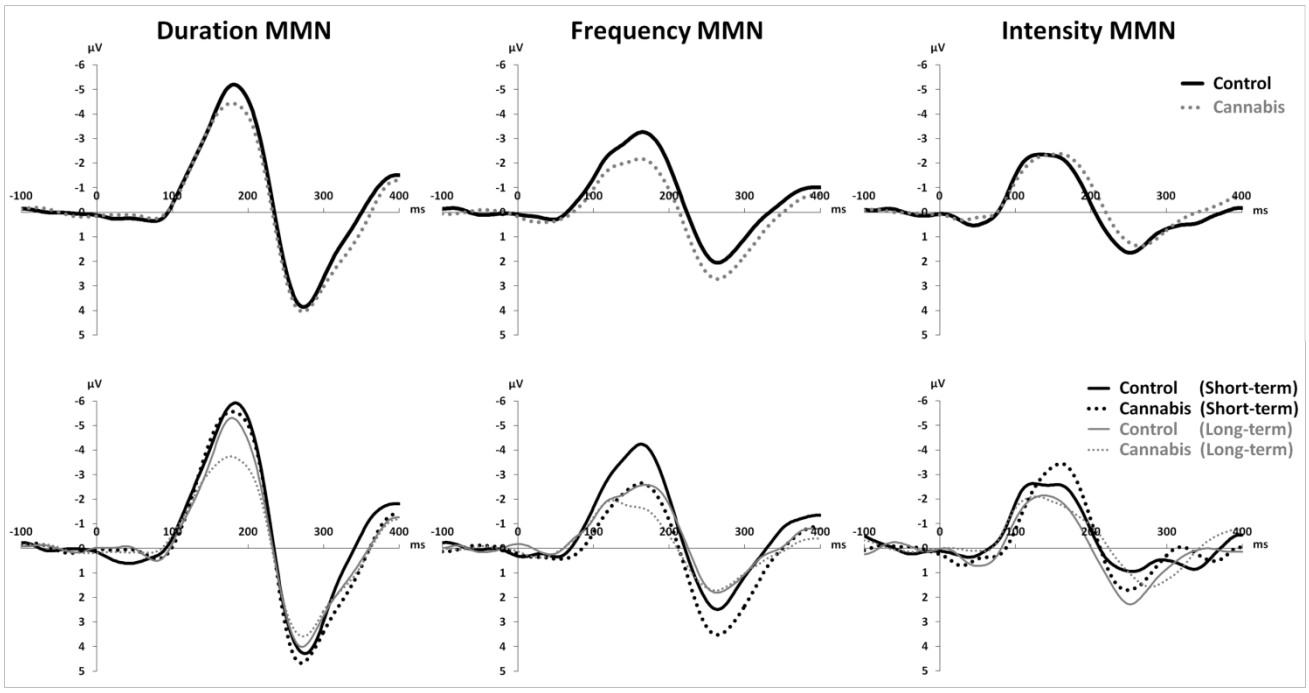

Duration MMN
Frequency MMN
Intensity MMN
μV
ms
Control
Cannabis
Control (Short-term)
Cannabis (Short-term)
Control (Long-term)
Cannabis (Long-term)

Figure 2.

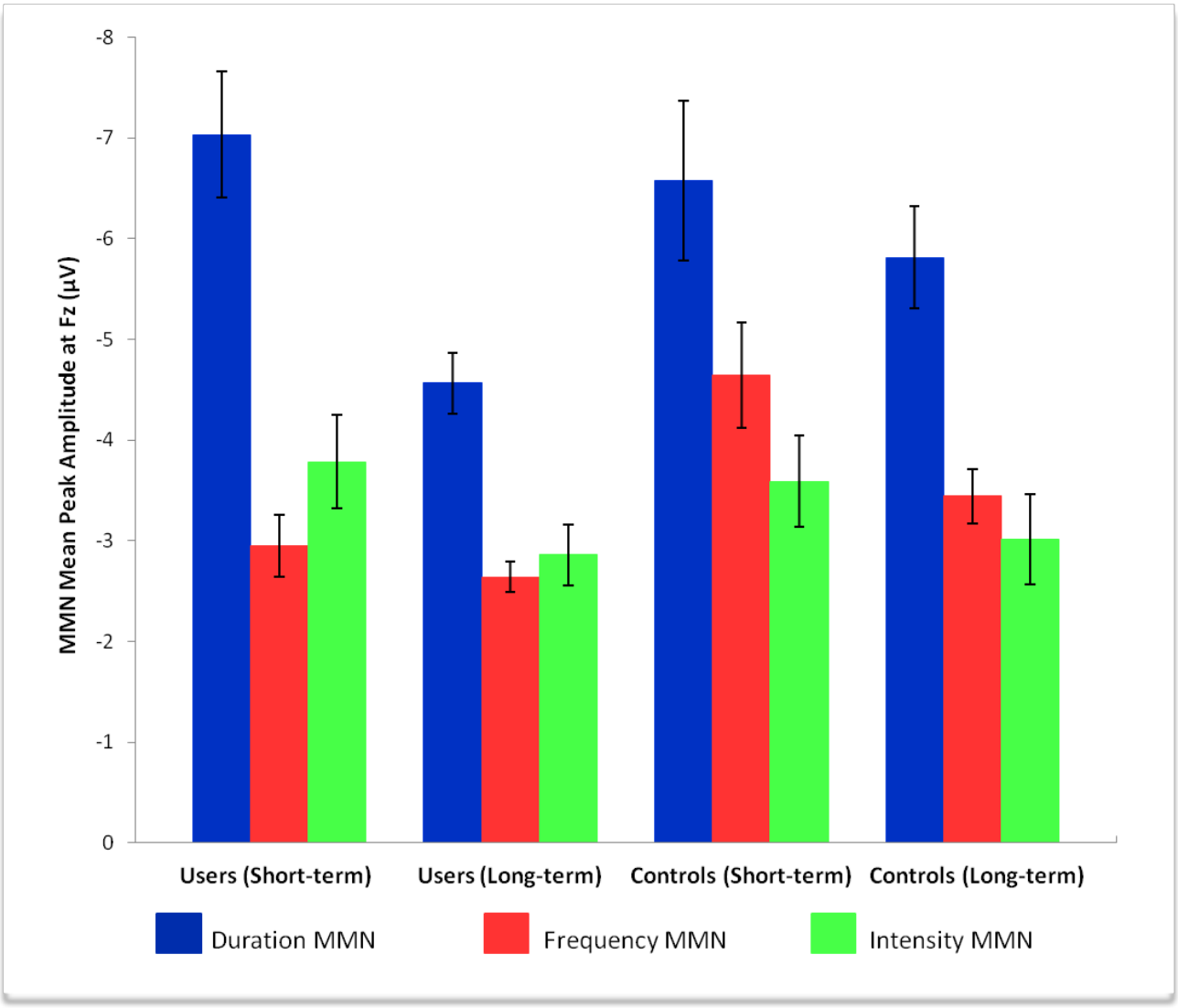

MMN Mean Peak Amplitude at Fz (μV)
-8
-7
-6
-5
-4
-3
-2
-1
0
Users (Short-term)
Users (Long-term)
Controls (Short-term)
Controls (Long-term)
Duration MMN
Frequency MMN
Intensity MMN

Figure 3.

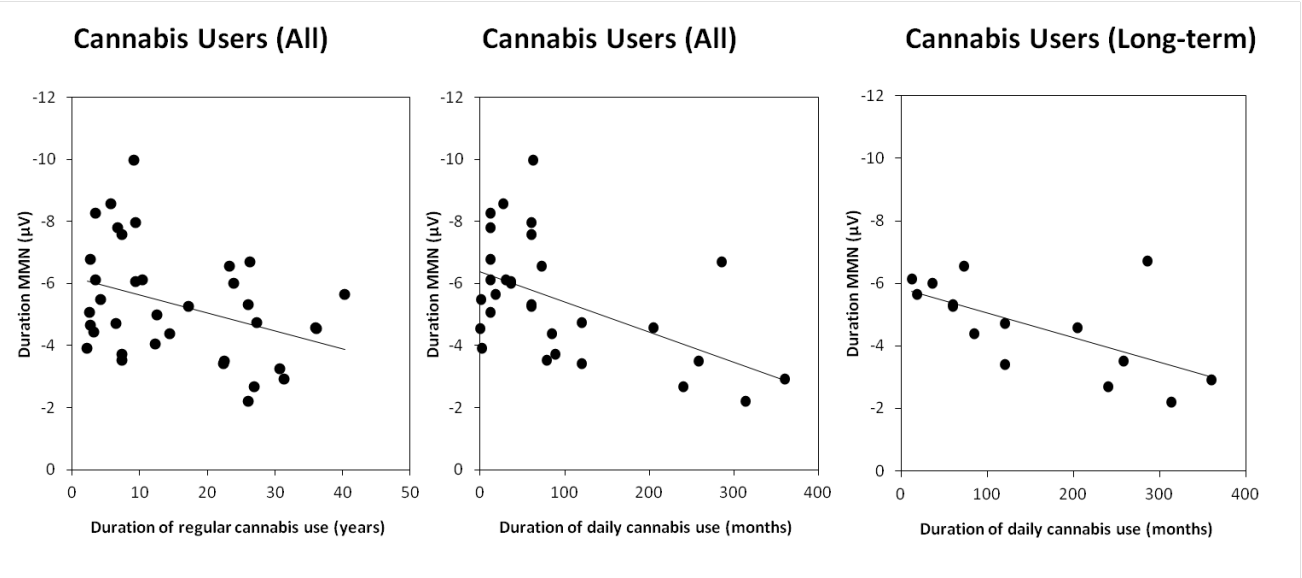
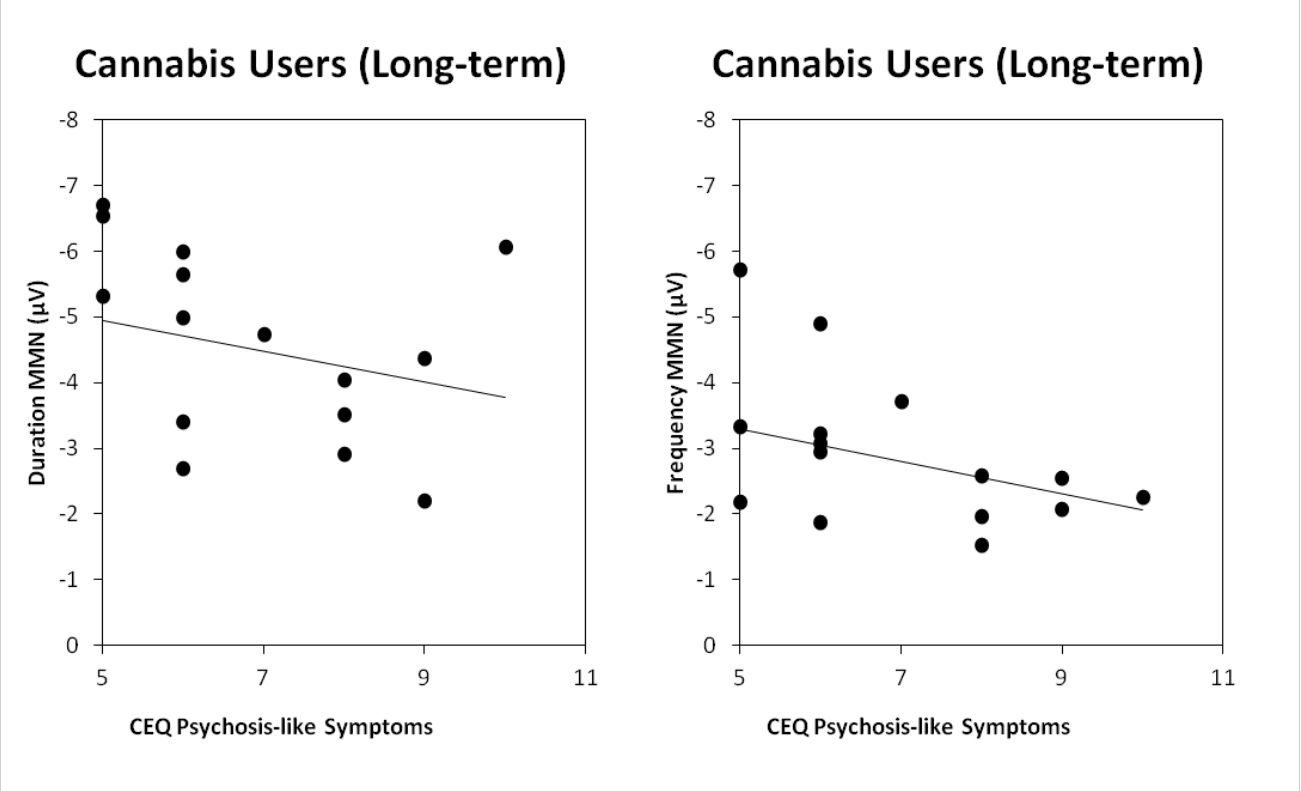

Cannabis Users (All)
Duration MMN (μV)
-12
-10
-8
-6
-4
-2
0
0
10
20
30
40
50
Duration of regular cannabis use (years)
Cannabis Users (All)
Duration MMN (μV)
-12
-10
-8
-6
-4
-2
0
0
100
200
300
400
Duration of daily cannabis use (months)
Cannabis Users (Long-term)
Duration MMN (μV)
-12
-10
-8
-6
-4
-2
0
0
100
200
300
400
Duration of daily cannabis use (months)

Figure 4.


Cannabis Users (Long-term)
Duration MMN (µV)
-8
-7
-6
-5
-4
-3
-2
-1
0
5
7
9
11
CEQ Psychosis-like Symptoms
Cannabis Users (Long-term)
Frequency MMN (µV)
-8
-7
-6
-5
-4
-3
-2
-1
0
5
7
9
11
CEQ Psychosis-like Symptoms

Chronic Effects of Cannabis Use on the Auditory Mismatch Negativity

Supplement 1

Exclusion Criteria, Hearing and Handedness Characteristics

Participants were excluded if they had a current psychiatric diagnosis, neurological or other medical disease, disorder or injury that would interfere with electroencephalograph (EEG) testing, regular poly-substance use within the past 2 years (weekly for > 6 months), or used any illicit substance other than cannabis in the past month. Conditions that excluded for interfering with EEG testing were any head injury that resulted in unconsciousness, prolonged hospitalisation, surgery or rehabilitation; self-reported significant hearing loss; experiencing fits, convulsions, epileptic seizures, stroke, brain tumor, meningitis, encephalitis, multiple sclerosis, or being positive for HIV. All participants had hearing in the normal range (i.e. ≤ 25 dB) at 1000 Hz and 1500 Hz (determined via audiometric testing). Handedness was assessed by the Edinburgh Handedness Inventory (1).

Poly-substance Use, First Degree Relatives with Psychosis and Participants at Potential Risk for Psychiatric Conditions

Poly-substance use was minimal in the final sample: two cannabis users reported regular amphetamine use more than 18 years ago and two others more than 2 years ago. Two participants reported one occasion of ecstasy use in the month prior to testing, but no amphetamine derivatives were detected in their urines. Their data were retained in the analysis. Three controls and two users (one short- and one long-term) had first degree relatives with schizophrenia; none of their data (mismatch negativity (MMN), clinical or symptomatic) were found to differ from their respective groups in any way, and hence these participants were retained in the analyses (which were also unaltered by their exclusion). Exclusion of six cannabis users with K10 scores above the mild range did not alter the pattern of significant results for MMN comparisons between users and controls. Similarly, exclusion of five short-term users and one long-term user with K10

scores above the mild range retained significance for all sub-group comparisons of short- and long-term users and with their respective matched controls, other than for duration MMN between long-term users and their matched controls, which was still significantly smaller in long-term users relative to short-term users (p = .003).

EEG Data Recording and Processing

EEG data were recorded using an electrode cap with 19 Ag/AgCl electrodes (international 10-20 system), as well as electrodes on left and right mastoids, above and below the left eye and on the outer canthus of each eye. Data were referenced to the nose and grounded midway between Fz and Fpz. Impedances were below 10Ωk at the start of recording and sampled continuously at 500 Hz, with a bandpass filter of 0.1 – 100 Hz.

EEG data were analysed offline using Neuroscan software (Scan 4.4). Data were re-referenced to the average of the mastoids (2), electro-oculogram corrected (3), and epoched 100 ms pre-stimulus to 400 ms post-stimulus. Epochs were filtered at 1 Hz – 30 Hz (24 dB, zero-phase shift), baseline corrected to the pre-stimulus interval and then rejected if EEG signals exceeded ± 50 µV.

Outlier Analysis

All analyses were performed using SPSS (version 19). MMN data outliers (±1.5 times the inter-quartile range) were identified in long- and short-term user groups and their matched controls in each deviant condition and were excluded from the repeated measures analysis of variance (ANOVA) and univariate analyses as follows: 2 long-term users for frequency MMN, and 2 controls in the short-term age-matched control group for intensity MMN.

Post Hoc Comparisons to Decompose Two-Way (Condition by Cannabis Use Group) and Three-Way (Condition by Cannabis Use Group by Matched Duration Group) Interactions

To determine whether the differences between MMN deviant types differ between groups, separate 2 group x 2 deviant type ANOVAs were conducted as three pair-wise comparisons of deviant types (duration versus frequency, duration versus intensity and frequency versus intensity). Bonferroni adjustment to control for type-1 error (evaluating alpha at 0.05/3 = 0.0167) was used for each of the group comparisons: cannabis users and controls overall, short- and long-term cannabis user groups with each other and with their respective matched control subgroups.

The cannabis users versus controls overall group difference for frequency MMN was greater than that for intensity MMN [$F_{(1,74)}$ = 7.98, p = .006], while group differences did not differ for frequency and duration MMN [$F_{(1,76)}$ = 2.92, p = .09] or duration and intensity MMN [$F_{(1,76)}$ = 0.09, p = .76].

The group difference between long- and short-term users for duration MMN was significantly greater than that for frequency [$F_{(1,35)}$ = 11.58, p = .002] and intensity MMN [$F_{(1,37)}$ = 6.63, p = .014] but did not differ between frequency and intensity MMN [$F_{(1,35)}$ = 1.86, p = .18]. The group difference for frequency MMN in short-term users versus their matched controls was greater than that for intensity [$F_{(1,37)}$ = 5.97, p = .019] and duration MMN [$F_{(1,39)}$ = 7.70, p = .008] and did not differ between duration and intensity MMN [$F_{(1,37)}$ = 1.47, p = .23]. In the long-term users, the group difference relative to their matched controls for duration MMN was greater than that for intensity MMN [$F_{(1,37)}$ = 7.87, p = .008] but was not different from frequency MMN [$F_{(1,35)}$ = 1.22, p = .28] and frequency and intensity MMN group differences did not differ [$F_{(1,35)}$ = 1.89, p = .18].

Correlations Between MMN and Alcohol Use

Duration and intensity MMN were found to correlate with frequency of alcohol use in long-term users (duration: *rho* = -.47, p = .045; intensity: *rho* = -.46, p = .049) and quantity of alcohol consumed per month correlated with intensity MMN amplitude (*rho* = -.46, p = .05). After controlling for alcohol use frequency, the association between duration of daily cannabis use and duration MMN in long-term users remained significant (partial r = .62, p = .019), and an association with intensity MMN emerged (partial r = .57, p = .04).

Correlations Between Cannabis Use and Psychosis-Proneness

Associations between psychosis-proneness and cannabis use measures were examined, independent of MMN. Analysis of the Cannabis Experiences Questionnaire in the overall cannabis user group, showed that the duration of cannabis use negatively correlated with paranoid symptoms (regular use *rho* = -.36, *p* = .03; since first tried *rho* = -.45, *p* = .006), indicating greater paranoid symptoms while intoxicated was associated with shorter duration of cannabis use. Hours since last use also correlated negatively with paranoid symptoms (*rho* = -.36, *p* = .03), after-effects (*rho* = -.35, *p* = .04) and psychosis-like symptoms (*rho* = -.41, *p* = .01), indicating greater endorsement of retrospective symptoms the more recently cannabis was used. In short-term users, greater quantity use was associated with less feelings of euphoria (*rho* = -.48; *p* = .045), and more recent use was associated with increased paranoid symptoms (*rho* = -.61, *p* = .007). Younger age of onset of regular use in short-term users was related to increased after-effects (*rho* = -.52, *p* = .03), amotivational (*rho* = -.47, *p* = .05) and psychosis-like symptoms (*rho* = -.60, *p* = .008). In long-term users, greater quantity of cannabis use correlated positively with greater after-effects (*rho* = .49, *p* = .041), with a trend also for greater amotivational symptoms (*rho* = .44, *p* = .065).

Higher Community Assessment of Psychic Experiences (CAPE) total symptom frequency scores were associated with increased frequency and quantity of cannabis use per month in the overall cannabis user group (*rho* = .37, *p* = .021 and *rho* = .39, *p* = .02, respectively). In short-term users, greater CAPE total frequency score was associated with greater frequency of cannabis use and more recent use (*rho* = .58, *p* = .009 and *rho* = -.62, *p* = .005, respectively) whereas greater CAPE total distress due to symptoms was related to a younger age of onset of regular cannabis use and more recent use (*rho* = -.56, *p* = .013 and *rho* = -.46, *p* = .049, respectively). No associations between CAPE scores and cannabis use were identified in long-term users. No correlations were observed between Schizotypal Personality Questionnaire (SPQ) total scores and cannabis use in any group, and no psychosis-proneness measures correlated with alcohol or tobacco use.

Phenomenological Differences Between Short-Term Users and Long-Term Users

We observed a number of differences between short- and long-term users, and between these user groups and their respective matched controls. Short-term users smoked more cigarettes per day and had higher Beck Depression Inventory (BDI), Spielberger State-Trait Anxiety Inventory (State), and SPQ scores and marginally higher CAPE total frequency scores than their matched controls (Table 1). Long-term users had higher BDI scores, smoked more cigarettes per day and consumed more alcohol more frequently than their matched controls (Table 1). More pertinent to our investigation, short-term users exhibited higher psychosis-proneness scores (CAPE total frequency and SPQ total scores) than their matched controls, and when intoxicated experienced greater paranoia and reduced euphoria compared to long-term users (see Table 1). Short-term users were more symptomatic on measures of psychosis-proneness than long-term users and their symptoms were more closely associated with cannabis use measures, such as quantity, frequency, recency of use and age of onset of use. One possible explanation for this difference is exposure to more potent forms of cannabis at a younger age than the long-term users, due to the increasing Δ^9-Tetrahydrocannabinol but decreasing cannabidiol content of cannabis preparations over recent decades (4-6). However, it should be noted that other than duration of use, short- and long-term users did not differ on any other cannabis use measures.

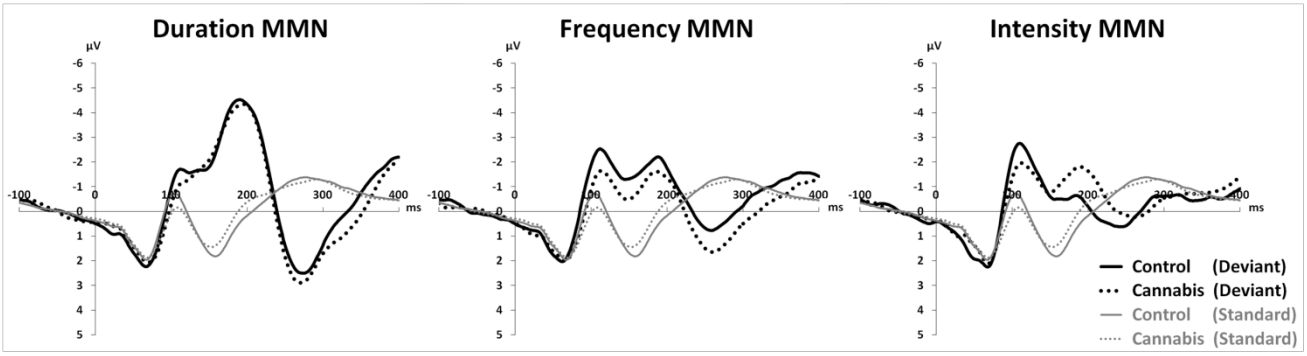


Figure S1. Mastoid referenced data are displayed for standard and deviant tones at Fz in each condition for cannabis users and controls. MMN, mismatch negativity.

Supplemental References

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5. Potter DJ, Clark P, Brown MB (2008): Potency of delta 9-THC and other cannabinoids in cannabis in England in 2005: implications for psychoactivity and pharmacology. *J Forensic Sci.* 51:90-94.
6. Mehmedic Z, Chandra S, Slade D, Denham H, Foster S, Patel AS, et al. (2010): Potency trends of Delta9-THC and other cannabinoids in confiscated cannabis preparations from 1993 to 2008. *J Forensic Sci.* 55:1209-1217.