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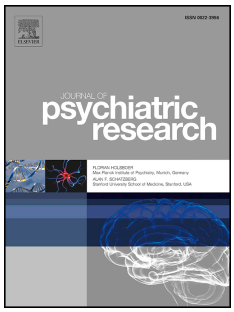

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Delayed bipolar and ganglion cells neuroretinal processing in regular cannabis users : the retina as a relevant site to investigate brain synaptic transmission dysfunctions

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ABSTRACT

Cannabis use is widespread worldwide, but the impact of smoking cannabis regularly on brain synaptic transmission has only been partially elucidated. The retina is considered as an easy means of determining dysfunction in brain synaptic transmission. The endocannabinoid system is involved in regulating retinal synaptic transmission, which might also be affected by tobacco. Previous preliminary results have shown impairments in retinal ganglion cell response in cannabis users. Here, we test the extent to which earlier retinal levels—bipolar cells and photoreceptors—are affected in cannabis users, i.e. by the association of tobacco and cannabis.

We recorded pattern (PERG) and flash (fERG) ERG in 53 regular cannabis users and 29 healthy controls. Amplitude and peak time of P50 and N95 (PERG) and of a- and b-waves (fERG) were evaluated. Cannabis users showed a significant increase in PERG N95 peak time and in fERG light-adapted 3.0 b-wave peak time, compared with controls (p=0.0001 and p=0.002, respectively; Mann-Whitney U test). No significant difference was found between the groups in terms of wave amplitude (p=0.525 and p=0.767 for the N95 and light-adapted 3.0 b-wave amplitude respectively; Mann-Whitney U test). The results demonstrated delayed ganglion and bipolar cell responses in cannabis users. These results reflect a delay in the transmission of visual information from the retina to the brain. This retinal dysfunction may be explained by an effect of cannabis use on retinal synaptic transmission. Main limitations of these results concern tobacco and alcohol use that differed between groups. The consequences of these anomalies on visual perception along with the molecular mechanisms underlying this retinal dysfunction should be explored in future human and animal studies.

Keywords: cannabis, endocannabinoid system, retina, retinal information processing, electroretinogram, synaptic transmission

Introduction

Regular cannabis use is a critical public health challenge, since cannabis is an addictive drug and one of the most frequently used in industrialized countries (Degenhardt et al., 2008). Cannabis is known to act on several brain synaptic transmission signaling pathways as well as tobacco (Bossong and Niesink, 2010). However, it is difficult to directly access the functioning brain and determine the long-term modulation of brain synaptic transmission following regular cannabis use. Indirect investigations are therefore needed. The retina is a particularly relevant means of access for studying the impact of regular cannabis use on brain synaptic transmission, because it is an anatomical and developmental extension of the central nervous system (CNS), previously suggested as being a good site for indirectly investigating the functioning brain in psychiatric and addictive disorders (Lavoie et al., 2014b, 2014a, Schwitzer et al., 2015a, 2016b, 2017b). Like the brain, the retina is organized in layers of specialized neurons interconnected by synapses (Hoon et al., 2014). These retinal neurons share several anatomical and functional properties with brain neurons (Hoon et al., 2014). For example, dopaminergic, serotoninergic, glutamatergic, cholinergic and GABAergic neurotransmitters are key molecules for retinal synaptic transmission. Moreover, the human retina has a functional endocannabinoid system, which is detected in rod and cone photoreceptors and bipolar and ganglion cells (Schwitzer et al., 2015b, 2016a; Yazulla, 2008). Animal studies have shown the endocannabinoid system to be involved in regulating the release of neurotransmitters such as dopamine, serotonin, noradrenaline, glutamate and γ-aminobutyric acid (GABA) in photoreceptors and bipolar and ganglion cells (Schwitzer et al., 2015b, 2016a; Yazulla, 2008). Additionally, an experimental study in CB2 knockout mice showed changes in the fERG a- and b-wave in both scotopic and photopic conditions (Cecyre et al., 2013), suggesting that cannabinoid receptor activation due to cannabis would lead to changes in photoreceptor and bipolar cell function. Such effects may be aggravated by the

intake of tobacco together with cannabis, related to its cholinergic effects, but also indirect vascular effects.

Retinal neuron function can be assessed objectively using an electroretinogram (ERG) (Holder et al., 2010). ERGs record the light-evoked electric potential originating from the retina in response to different types of stimulus (Holder et al., 2010). The recorded retinal response reflects retinal neuron signaling and is associated with changes in levels of neurotransmitters through the retina (Hoon et al., 2014). Using flash light stimuli, the flash ERG (fERG) evaluates the rod, bipolar cell and cone functions (McCulloch et al., 2015). Using alternative black and white checkerboards, the pattern ERG (PERG) evaluates ganglion cell function (Bach et al., 2013; Porciatti, 2015). Standardized protocols are available for clinical settings and research to ensure reproducible results (Bach et al., 2013; McCulloch et al., 2015). Typical fERG and PERG traces are presented in Figure 1. Using PERG in a preliminary study, our group has recently shown a delay in the transmission of action potentials by the retinal ganglion cells in regular cannabis users compared with controls. More specifically, there was an increase in N95 peak time (Schwitzer et al., 2017a). This effect was suggested to be independent of alcohol consumption. It is now crucial to 1) confirm our findings obtained in the preliminary analysis on the total number of patients originally planned in the Causamap study, 2) investigate whether earlier retinal stages are also altered in regular cannabis users to precise where the delay of information processing is located into the retina, 3) evaluate the specificity and sensitivity of the potential functional retinal abnormalities

The aim of this study was to verify whether early retinal stages, involving in particular bipolar and photoreceptor cells, are altered in cannabis users. Given the role of the cannabinoid system in regulating neurotransmitter release in retinal photoreceptors and bipolar and ganglion cells, we hypothesized that dysfunctions may be observed in regular cannabis users at both early and late stages of retinal processing.

Material and methods

Population and ethics statement. Regular cannabis users (n=53) and matched healthy drug-naive controls (n=29) were recruited among the general population via a special press campaign and data were collected from February 11, 2014, to June 30, 2016. Prior to taking part in the study, volunteers provided their detailed psychoactive drug and medical history, underwent a full psychiatric evaluation, and signed consent forms detailing all aspects of the research. All participants received payment in the form of €100 in gift vouchers. The study protocol met the requirements of the Helsinki Declaration and was approved by the Ethics Committee of Nancy University Hospital. This study is part of a bigger project, Causa Map, which is researching the impact of regular cannabis use on the visual system. All participants also underwent neuropsychological assessments and EEG was recorded while performing several visual tasks.

Inclusion criteria, clinical and biological assessments. The inclusion criteria for the cannabis group were regular cannabis use equivalent to an average of at least 7 cannabis consumptions per week over the past month. The total years of cannabis use varied between 5 and 14 years with a median at 7. Others inclusion criteria included a positive urine toxicology screen for tetrahydrocannabinol (THC) metabolites, no other illicit substance use in the past month, a negative urine toxicology screen for other illicit substances, and no DSM-IV diagnosis of Axis I disorders. Since tobacco is regularly mixed with cannabis in joints, cannabis users may meet the criteria for tobacco dependence according to the Fagerström test. Cannabis users were required to have abstained from cannabis use for at least 12 hours to avoid acute cognitive dysfunction caused by cannabis use. The inclusion criteria for the healthy control subjects were no history of illicit substance use, a negative urine toxicology screen for THC metabolites and the other illicit drugs tested, and no history of DSM-IV

diagnosis of Axis I psychiatric disorders. All participants were aged 18 to 35 years, had no history of neurological disease, no family history of schizophrenia or bipolar disorders, and were medication-free except for oral contraceptives in the case of women. They had no history of ophthalmological disease except for corrected refractive errors. All fared normally in an ophthalmic evaluation, which included visual acuity and a fundoscopic examination. Importantly, visual acuity measured with the Monoyer scale was at least 10/10 in each eye for all participants. None of the participants reported visual symptoms, and none was found to have any media opacities. If participants reported alcohol dependence based on their score in the Alcohol Use Disorders Identification Test (AUDIT) they were excluded from the study. The Mini-International Neuropsychiatric Interview (M.I.N.I.) was used to assess current and past history of psychiatric diseases and substance use. In addition, the Cannabis Abuse Screening Test (CAST), Fagerström test and AUDIT were performed to assess use, abuse and dependence with respect to cannabis, tobacco and alcohol respectively. The extent of cannabis use was clinically assessed in an interview and a questionnaire as follows: age when regular cannabis use began, total years of cannabis use, average number of joints smoked daily and weekly over the past month and average number of grams smoked weekly (Table 1). In order to obtain objective confirmation of cannabis consumption, urine drug screens (nal von minden, Moers, Germany) were performed for cannabis, buprenorphine, benzodiazepines, cocaine, opiates, amphetamines and methadone immediately before electroretinogram testing.

Experimental protocol

PERG and fERG were performed according to the International Society for Clinical Electrophysiology of Vision (ISCEV) standards for PERG and fERG (Bach et al., 2013; McCulloch et al., 2015). The MonPackONE system (Metrovision, Pérenchies, France) was used for stimulation, recording and analysis. Electrical signals were recorded simultaneously

from both eyes. Averaged retinal responses were first obtained from each eye and then values of parameters -peak time and amplitude- were averaged over both eyes for analysis. Electrical signals were recorded on non-dilated (PERG) and dilated pupils (fERG, Tropicamide 0.5%), with DTL electrodes (Metrovision, Pérenchies, France) placed at the bottom of the conjunctival sac. The pupil's size was noted before and after fERG recordings and remained systematically constant during the whole testing period. Ground and reference electrodes were attached to the forehead and external canthi.

Pattern electroretinogram (PERG) measurements

A black and white contrast reversible checkerboard, with 0.8° check size, 93.3% contrast level, 100 candela/m² constant luminance white area, and 4 reversals per second was used. The participants were positioned one meter from the screen. In the case of participants with refractive disorders, an appropriate optic correction was provided. At least 220 responses were recorded for each participant, with constant ambient room-lighting to achieve the best signal-to-noise ratio.

Flash electroretinogram (fERG) measurements

fERG recordings were performed in dark and light conditions. Participants were positioned 30 centimeters from the screen. They were dark-adapted for a period of 20 minutes before dark-adapted fERG were recorded. They were then light-adapted for 10 minutes to a light background set at 30 candela/m² (cd/m²) managed by the MonPackONE system before light-adapted fERG was performed. At least 8 and 16 responses, for dark- and light-adapted ERG respectively, were recorded for each participant. Each retinal response is called according to the strength of the flash in candela.m².s^{-1}. To assess the functioning of the rod and cone

system separately, dark-adapted 0.01 ERG and light-adapted 3.0 ERG were performed respectively.

Analysis

PERG and fERG data were analyzed with an ophthalmic monitor (Metrovision, Pérenchies, France). Analysis was performed with the experimenter blind to the status of the subject being recorded (cannabis user or control). Two main components are usually described on a typical PERG trace: an electropositive component, P50, followed by an electronegative component, N95. N95 is believed to reflect the response of retinal ganglion cells. P50 reflects the response of the retinal ganglion cells and macular photoreceptors and is used to evaluate the macular function. Two main parameters are derived from P50 and N95, known by convention as the amplitude measured in microvolts (µV) and the peak time (i.e. latency) measured in milliseconds (ms). N95 amplitude is measured from the trough of the N95 to the peak of the P50. P50 amplitude is measured from the trough of the inconstant N35—or from the baseline—to the peak of the P50. Peak time denotes the time taken to reach the maximum N95 and P50 amplitudes. Conversely, the two main components usually described on a typical fERG are an electronegative component, a-wave, followed by an electropositive component, b-wave. The a-wave is not detected in the dark-adapted 0.01 ERG response because it is masked by the b-wave. An a-wave is attributed to the retinal photoreceptors and a b-wave is attributed to the retinal bipolar cells, postsynaptic to photoreceptors. Two main parameters are derived from a- and b-waves, known by convention as the amplitude measured in microvolts (µV) and the peak time measured in milliseconds (ms). a-wave amplitude is measured from the baseline to the trough of the a-wave. b-wave amplitude is measured from the trough of the a-wave to the peak of the b-wave. Peak time denotes the time taken to reach

the maximum a- and b-wave amplitudes. Typical traces of PERG and fERG are presented in Figure 1.

Statistical analysis

Depending on the non-parametric distribution of several variables included in the analyses, a Mann-Whitney U test, Chi-square test and Spearman's rank correlation test were used when appropriate to compare the two cannabis user/control groups or to test the association between variables. A logistic regression was performed to examine the association between the binary dependent cannabis user/control variable and the independent variables that were significant between cannabis users/controls in univariate analysis and uncorrelated. Regarding correlated variables, the most significant between cannabis users and controls was retained in the logistic regression. Regression lines were used to analyze the interaction graphically. A receiver operating characteristic (ROC) was applied to the values of the independent variables that were significant to estimate the sensitivity and specificity of cut-off values between regular cannabis users and controls. We used a conservative level of significance in comparison with alpha <0.015%. Statistical analyses were performed using IBM-SPSS Statistics 22.0 (IBM corp.).

Results

Demographic and substance use characteristics

The demographic and substance use characteristics of the participants are described in Table 1. There was no relevant difference between controls and cannabis users in terms of age (p=0.517) or gender (p=0.618), but differences were noted between groups in terms of years of education (p=0.0001; lower in cannabis users) and alcohol use (higher in cannabis users; p=0.0003 for average alcohol consumption/week; p=0.0001 for AUDIT score). Because tobacco is widely mixed with cannabis in joints, 44 in 53 cannabis users were also tobacco smokers, whereas all the controls were non-smokers. According to the Fagerström test, 27 in 53 cannabis users were not dependent on tobacco, 12 in 53 were slightly dependent, 4 in 53 were mildly dependent and 1 in 53 was highly dependent.

Pattern electroretinogram (PERG) parameters: N95 and P50

The median and interquartile range of the N95 peak time was 95.5 ms [91.8: 99.9] in cannabis users versus 88.9 ms [84.5: 91.1] in controls. This difference was significant between groups (p=0.0001; Mann-Whitney U test) (Figure 2). There was no significant difference between groups for N95 amplitude, P50 peak time and P50 amplitude (Table 2).

Full-field electroretinogram (fERG) parameters

Dark-adapted 0.01 ERG

There was no significant difference between groups in terms of b-wave amplitude and peak time (Table 2).

Light-adapted 3.0 ERG

The median and interquartile range of the b-wave peak time was 36.3 ms [35.8: 37.2] in cannabis users versus 35.8 ms [35.1: 36.3] in controls. This difference was significant between groups (p=0.002; Mann-Whitney U test) (Figure 3). There was no significant difference between groups for b-wave amplitude and a-wave amplitude and peak time (Table 2).

Logistic regression on 3.0 ERG b-wave peak time and N95 peak time

In order to analyze alcohol consumption and ERG parameters simultaneously and due to the significant differences in univariate analysis between cannabis user/control groups in terms of AUDIT score, average alcohol consumption/week, light-adapted 3.0 ERG b-wave peak time and N95 peak time, we conducted a logistic regression to test the association between them and cannabis users/controls as the binary outcome variable. Average alcohol consumption/week was removed due to the significant correlation (Spearman rank correlation (SCR)=0.720: p=0.0001) with the AUDIT score, which is more significant. There is no significant correlation between the AUDIT score, light-adapted 3.0 ERG b-wave peak time and N95 peak time (SCR=0.107: p=0.337 for AUDIT score *vs* N95 peak time; SCR=0.113: p=0.312 for AUDIT score *vs* light-adapted 3.0 ERG b-wave peak time and SCR=0.177: p=0.111 for N95 peak time *vs* light-adapted 3.0 ERG b-wave peak time).

Results of the logistic regression (N=82; LR Chi-square=49.81; p=0.0001; Hosmer-Lemeshow Chi-square=10.42; p=0.237; 87.80% of subjects classified correctly in their respective group: 90.6% (48/53) of cannabis users and 82.8% (24/29) of controls) showed that the N95 peak time, AUDIT score and light-adapted 3.0 ERG b-wave peak time were still significant (Wald p=0.0001; Wald p=0.001; Wald p=0.010 respectively). The AUDIT score×N95 peak time and AUDIT score×light-adapted 3.0 ERG b-wave peak time products

(interactions) were not added to the model because they are too strongly correlated with the AUDIT score (SRC= 0.993: p=0.0001; SRC= 0.995: p=0.0001 respectively). We thus investigated these interactions graphically, for N95 peak time and for light-adapted 3.0 ERG b-wave peak time respectively, with regression lines on the AUDIT score for controls and for cannabis users. Concerning N95 peak time and the AUDIT score, the 95% confidence intervals of the two slopes, which are both negative, overlap and the lines do not cross among the ranges of the observed values (controls: -0.479; [-1.285; 0.328]; cannabis users: -0.144; [-0.625; 0.337]) (Figure 4). Concerning light-adapted 3.0 ERG b-wave peak time, the 95% confidence intervals of the two slopes, which are both negative, overlap and the lines do not cross among the ranges of the observed values (controls: -0.023; [-0.158; 0.112]; cannabis users: -0.014; [-0.087; 0.060]) (Figure 5).

Correlations

We conducted correlations between the ERG parameters (N95 peak time, light-adapted fERG 3.0 b-wave peak time), education level and alcohol consumption (AUDIT score). The correlations were evaluated in the whole sample of subjects as well as in each group. None of these correlations was significant at a level of 0.015.

Sensitivity and specificity of light-adapted 3.0 ERG b-wave peak time and N95 peak time

An ROC was used to assess the best cut-off value of N95 peak time and of light-adapted 3.0 ERG b-wave peak time, capable of discriminating between cannabis users and controls. The results indicated that the cut-off value for N95 peak time giving a good balance between sensitivity and specificity for regular cannabis users and controls was 91.3 ms (Area under the curve (AUC)=0.83; 95% CI [0.73; 0.92]; p=0.0001). Six out of 29 controls are below the cut-off, with an estimated specificity of 79.3% (95% CI [0.62; 0.90]) whereas 11 out of 53 regular cannabis users are above the cut-off, with an estimated sensitivity of 79.2% (95% CI [0.67; 0.88]). The results indicate that the cut-off value for light-adapted 3.0 ERG b-wave peak time giving a good balance between sensitivity and specificity for regular cannabis users and controls was 36.1 ms (AUC=0.71; 95% CI [0.58; 0.83]; p=0.002). Twenty out of 29 controls are below the cut-off, with an estimated specificity of 69% (95% CI [0.51; 0.83]), whereas 38 out of 53 regular cannabis users are above the cut-off, with an estimated sensitivity of 71.7% (95% CI [0.58; 0.82]) (Figure 6).

Discussion

We found delayed retinal processing in regular cannabis users compared with controls in two critical stages, namely bipolar and ganglion cells. These results suggest a delay of approximatively 6 ms in the emission of action potentials by the retinal ganglion cells in cannabis users, shown by an increase in PERG N95 peak time. Another finding of this study is the delay observed in regular cannabis users in the response of cone bipolar cells—an earlier stage of retinal processing—shown by an increase in the b-wave peak time of the light-adapted 3.0 fERG. This result supports a delay in the gradual variation of membrane potential in cone bipolar cells of approximatively 0.5–1 ms in cannabis users in comparison with controls. No anomaly was observed in either rod and cone photoreceptors or in bipolar cells connected to the rod receptors.

We observed an increase in N95 and b-wave peak time. According to these findings, ganglion cells and bipolar cells of the cone system take longer to react to a light stimulation when under the influence of regular cannabis use. Moreover, anomalies in peak time occur with no change in amplitude, which suggests that the total number of cells involved in the visual response is preserved, but that their functional properties are impaired. The N95 anomalies confirm our earlier findings; the signal sent to the brain by the optic nerve formed by the axons of the ganglion cells is delayed. In addition, these results suggest that this delay exists already at an earlier retinal stage, i.e. at a post-receptoral level in the bipolar cells of the cone system. It seems to be amplified in ganglion cells by ≈6 ms. Although regular cannabis users did not report visual symptoms or visual deficits, these findings may imply that information is processed less rapidly, psychomotor retardation and attentional disorders, described commonly in regular cannabis users (Broyd et al., 2016). The retinal abnormalities are not correlated with clinical observations, but they could serve as early functional markers

of the impact of the combined use of cannabis and tobacco on brain synaptic transmission. Why P50 peak time is not altered worth to be discussed. This is probably due to the fact that the exact origin of this wave is not affirmed with certainty. P50 would be in part related to retinal ganglion cell function and to photoreceptors and bipolar cells function situated in the macula (Holder et al., 2010). Retinal impairments have already been proposed as indicators of neurological dysfunctions in CNS disorders (London et al., 2013). For example, in multiple sclerosis, Parkinson's disease and Alzheimer's disease, ganglion cell dysfunctions often precede brain dysfunctions and may constitute early markers of brain dysfunction (Celesia et al., 1986; Froehlich and Kaufman, 1993, 1994; Garcia-Martin et al., 2014; Holder et al., 2009; Krasodomska et al., 2010; Parisi et al., 2001; Peppe et al., 1995, 1998). In another hand, a significant reduction in retinal contrast gain measured with PERG measurements was found in unmedicated and medicated depressed patients independently of the antidepressant therapy, in comparison with the control group (Bubl et al., 2015, 2012, 2010).

When performing an ROC analysis on both N95 peak time and light-adapted 3.0 ERG b-wave peak time, we observed that the parameter capable of classifying both cannabis users and controls correctly in their corresponding group with the best specificity and sensitivity is the N95 peak time. In comparison with the ROC analysis performed in our preliminary study on the N95 peak time, we found that the cutoff value (91.3 ms vs 91.1 ms), sensitivity (79.2% vs 78.6%) and specificity (79.3% vs 75%) are noticeably similar and thus could give support to the reliability and reproducibility of the findings. It would be inappropriate, at this time of research, to use these data as markers to separate patients from controls in the general population. However, they can be viewed as an interesting trail to follow in order to study central neurotransmission dysfunctions in cannabis users.

Cannabis is a neuromodulator substance that acts directly and indirectly on several synaptic transmission signaling pathways, and especially on glutamatergic synaptic transmission (Bossong and Niesink, 2010). Glutamate is one of the key neurotransmitters detected in the retina and is known to be involved in the vertical transmission of the retinal signal from photoreceptors to ganglion cells (de Souza et al., 2013). Bipolar cells of the cone system and ganglion cells, which function less effectively in cannabis users, both have a functional cannabinoid system (Schwitzer et al., 2015b, 2016a; Yazulla, 2008). This system helps to regulate synaptic transmission in these cells. We suggest that tetrahydrocannabinol (THC) may alter synaptic transmission in these cells and delay the cellular response by acting directly on the cannabinoid receptors in bipolar and ganglion cells. Previous findings in humans and in animals support this hypothesis. Strong labeling of CB1 has been detected in human photoreceptors, whereas human bipolar and ganglion cells were moderately stained for CB1 (Straiker et al., 1999). Since bipolar and ganglion cells have lower levels of CB1 than photoreceptors, they may be more sensitive to the effect of THC on synaptic transmission. In mice retinal ganglion cells, the exogenous cannabinoid WIN 55212-2 induced a significant reduction in the frequency of spontaneous postsynaptic currents in retinal ganglion cells, through a presynaptic action on glutamatergic transmission (Middleton and Protti, 2011). These data speak in favor of delayed ganglion cell processing due to a cannabinoid agonist effect, which we have confirmed here in humans.

Following our previous preliminary study (Schwitzer et al., 2017a), we also evaluated the potential effect of alcohol consumption on our results. Delayed retinal responses remained significant when alcohol consumption was integrated into the statistical analysis. This suggests an isolated and independent effect of cannabis use on retinal function. Higher

alcohol consumption is common in regular cannabis users compared with controls (Meier et al., 2012). Alcohol and cannabis are two neuromodulator substances that act on CNS synaptic transmission signaling pathways. Therefore, when studying the effect of cannabis on CNS synaptic transmission, distinguishing its effect from the consequence of alcohol intake is crucial. Ideally, a control group of alcohol users would be useful to accurately evaluate the impact of alcohol consumption on retinal processing. The educational level was not integrated into the statistical analysis because it is most likely that it cannot alter the retinal functioning.

In addition to alcohol, tobacco is another substance that acts on CNS synaptic transmission and is consumed by regular cannabis users, particularly with cannabis in joints (Agrawal et al., 2012). Therefore, future studies should research this bias with a control group including tobacco smokers. The effect of chronic nicotine administration on ERG has not yet been evaluated. Dark-adapted and light-adapted fERG responses have been modified after acute nicotine administration in the form of gum 30 minutes before testing (Varghese et al., 2011), but the effect of regular tobacco use on fERG measurements still needs to be evaluated. Correlations performed in this study did not show an effect of tobacco on retinal function, but an indirect effect or an interaction with the effect of cannabis cannot be excluded. It remains a fact, though, that neuronal signaling is slowed down in cannabis users.

In summary, regular cannabis users showed slower retinal processing than the controls, a delay that stems from delayed bipolar and ganglion cell responses. Theses anomalies are underpinned by dysfunctions in retinal synaptic transmission caused by regular cannabis use. Molecular and genetic studies of the precise mechanisms underlying these retinal dysfunctions should be included in future research in this field. Since the retina is a

crucial site for investigation of brain synaptic transmission abnormalities in psychiatric and addictive disorders, these perspectives could help us understand the effects of cannabis on brain synaptic transmission. If brain synaptic dysfunctions are detected in the retina, these data could be particularly relevant because they may contribute to the development of pharmacotherapy for cannabis use disorder (CUD), for which there is no validated pharmacotherapy for CUD treatment.

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Figure legends:

Figure 1. Typical electroretinogram (ERG) traces obtained when assessing ganglion cell response with pattern ERG (PERG) (A), the response of the rod system with flash ERG (fERG) (B) and the response of the cone system with fERG (C). The arrows show how the parameters are measured, namely the P50, N95, a- and b-wave amplitude and peak time.

Figure 2. Dot plot of pattern electroretinogram (PERG) N95 peak time (ms) for cannabis users (n=53) and controls (n=29) with medians. Cannabis users showed increased peak time and the difference between the groups is highly significant (p=0.0001; Mann-Whitney U test).

Figure 3. Dot plot of flash electroretinogram (fERG) light-adapted 3.0 b-wave peak time (ms) for cannabis users (n=53) and controls (n=29) with medians. Cannabis users showed increased peak time and the difference between the groups is highly significant (p=0.002; Mann-Whitney U test).

Figure 4. Graphical investigation of the interaction between the pattern electroretinogram (PERG) N95 peak time and the AUDIT score. Linear regression lines of N95 peak time (ms) on the AUDIT score for controls (n=29) and for cannabis users (n=53). The 95% confidence intervals of the two negative slopes overlap and the lines do not cross among the ranges of the observed values (controls: -0.479; [-1.285; 0.328]; cannabis users: -0.144; [-0.625; 0.337]).

Figure 5. Graphical investigation of the interaction between the flash electroretinogram (fERG) light-adapted 3.0 b-wave peak time and the AUDIT score. Linear regression lines of fERG light-adapted 3.0 b-wave peak time (ms) on the AUDIT score for controls (n=29) and for cannabis users (n=53). The 95% confidence intervals of the two negative slopes overlap and the lines do not cross among the ranges of the observed values (controls: -0.023; [-0.158; 0.112]; cannabis users: -0.014; [-0.087; 0.060]).

Figure 6. Receiver operating characteristic (ROC) curves. A) The blue curve is related to N95 peak time. AUC=0.83; 95% CI [0.73; 0.92]; p=0.0001 for the cut-off value of 91.3 ms (6 out of 29 controls are below the cut-off, with an estimated specificity of 79.3% (95% CI [0.62;

0.90]) whereas 11 out of 53 regular cannabis users are above the cut-off, with an estimated sensitivity of 79.2% (95% CI [0.67; 0.88])). B) The green curve is related to light-adapted 3.0 ERG b-wave peak time. AUC=0.71; 95% CI [0.58; 0.83]; p=0.002 for the cut-off value of 36.1 ms (20 out of 29 controls are below the cut-off, with an estimated specificity of 69% (95% CI [0.51; 0.83]), whereas 38 out of 53 regular cannabis users are above the cut-off, with an estimated sensitivity of 71.7% (95% CI [0.58; 0.82]).

636 **Table legend:**

637 Table 1: Demographic and substance use characteristics of the participants

638 Table 2: Electroretinogram (ERG) parameters of the participants

639

Table 1. Demographic and substance use characteristics of the participants.

	Cannabis users (n= 53)	**Controls (n=29)**	***P*-value**
Gender (male/female) [a,d]	41 / 12	21 / 8	*p=0.618*
Age (years) [b,c]	23 (21 - 30)	24 (23 - 27)	*p*=0.517
Education (years) [b,c]	13 (12 - 14)	15 (14 - 16)	*p=0.0001*
Average number of alcohol uses/week [b,c]	4 (1,5 - 9)	1 (0 – 3,5)	*p=0.0003*
Alcohol Use Disorders Identification Test (AUDIT) scores [b,c]	7 (3,5 - 9)	3 (1 – 4,5)	*p=0.0001*
Fagerström Test scores [b] **(n=44)**	1 (0 - 3)	-	-
Average number of cigarettes/day [b]	4 (2 -10)	-	-
Age of first cannabis use [b]	16 (15 - 17)	-	-
Total years of cannabis use [b]	7 (5 - 14)	-	-
Average number of joints/week [b]	20 (14 - 30)	-	-
Cannabis Abuse Screening Test (CAST) scores [b]	4 (3 - 5)	-	-
Average number of grams of cannabis/week[b]	4,2 (3 - 10)	-	-

Categorical variable represented as frequencies [a]

Quantitative variable represented as median and interquartile range [b]

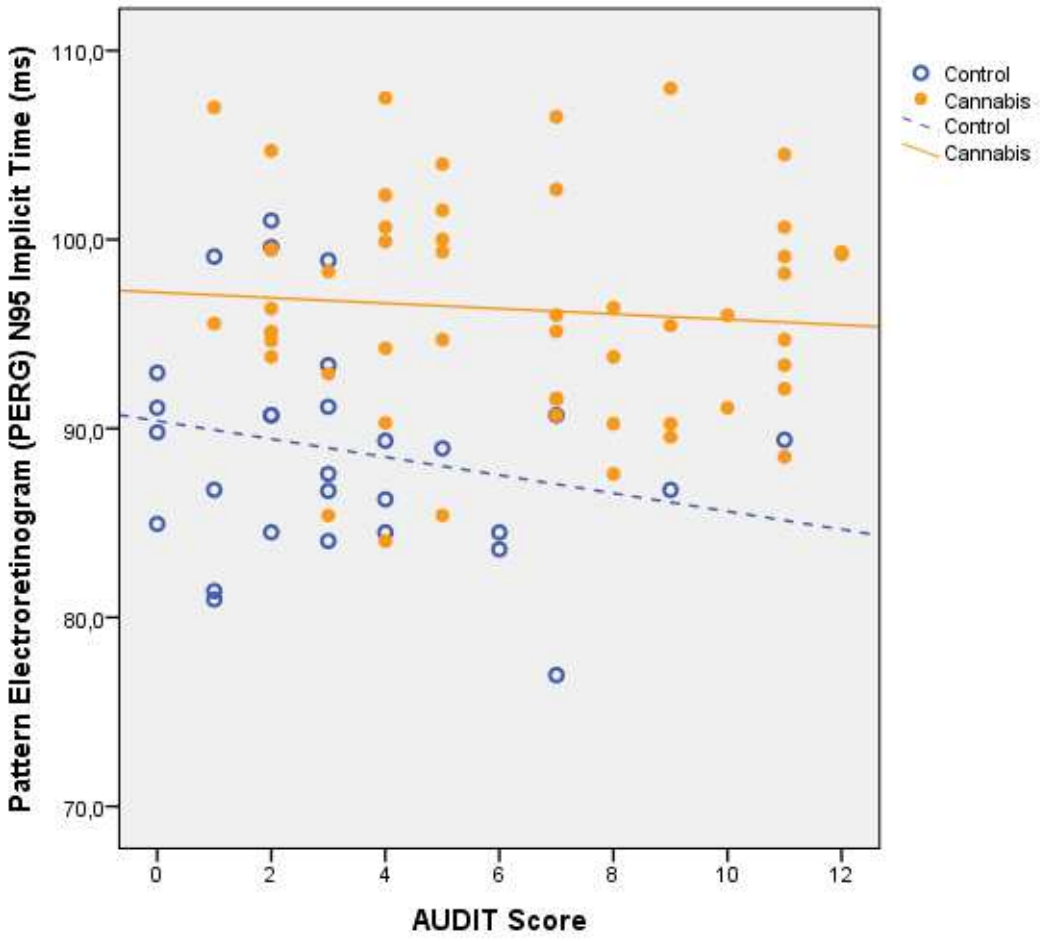
Mann-Whitney U test [c]

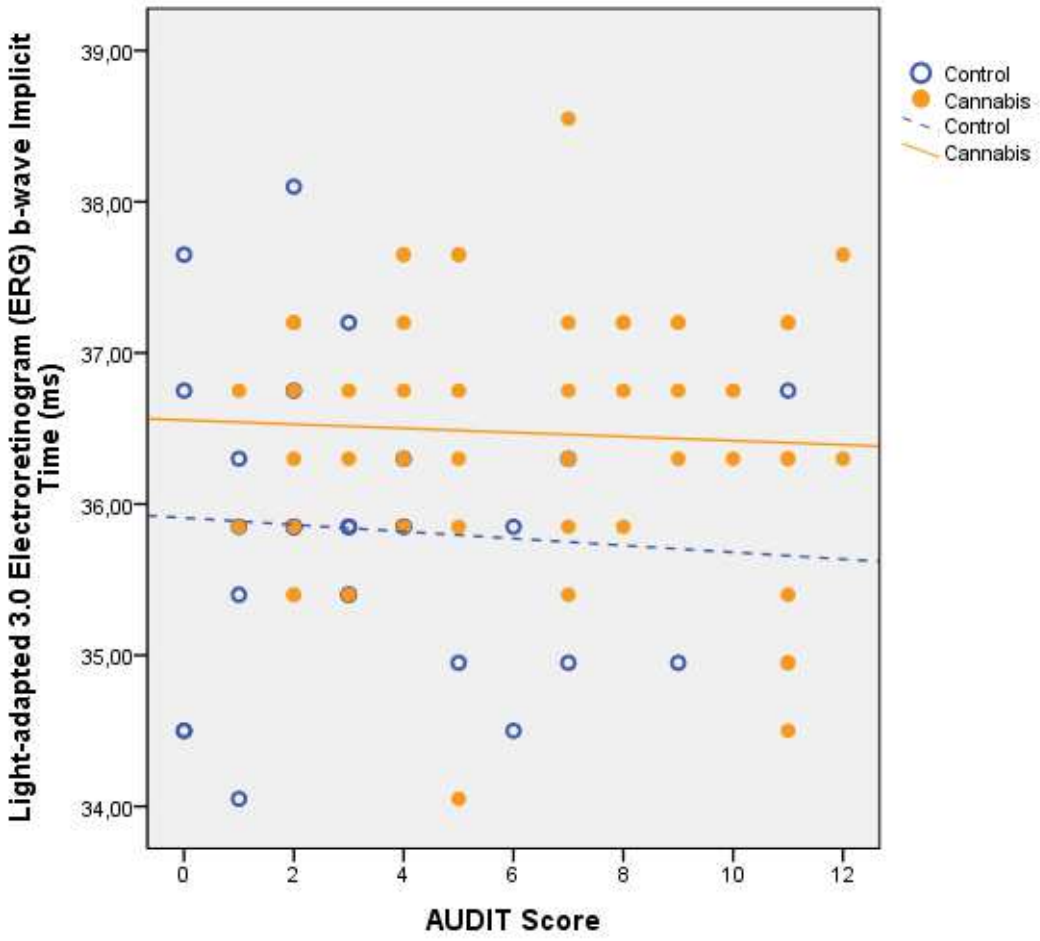
Chi-Square test [d]

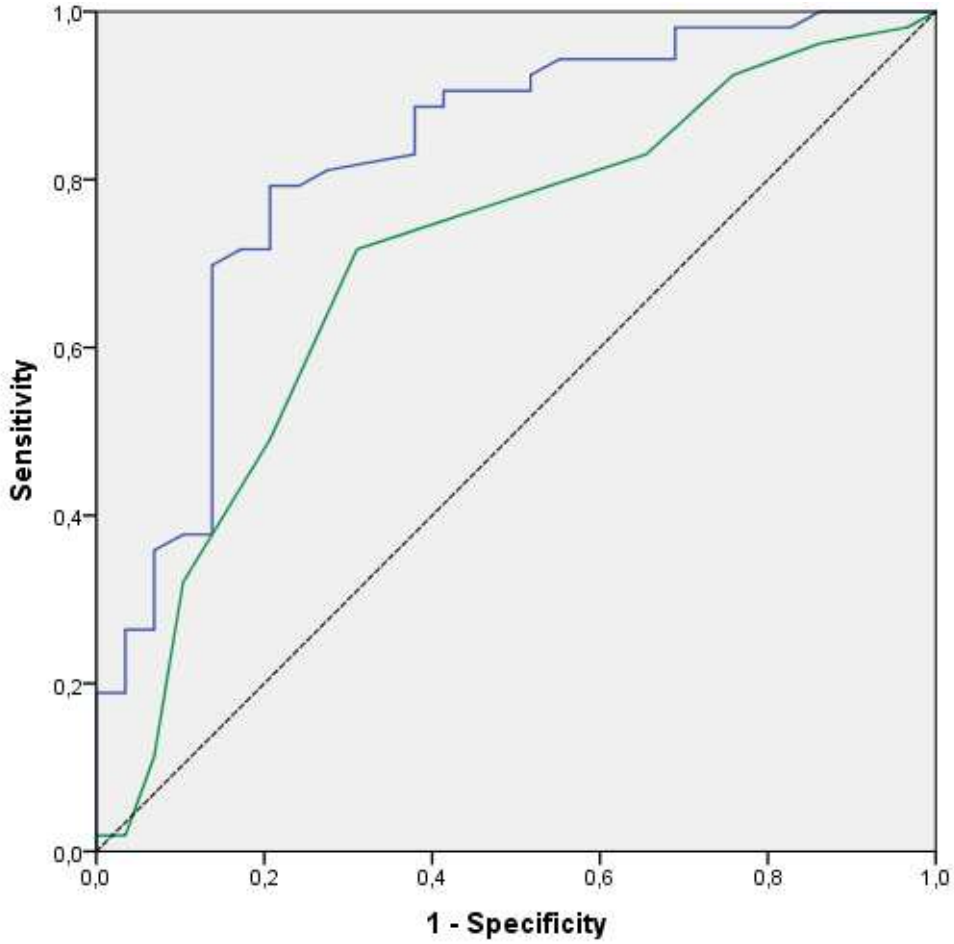
Table 2: Electroretinogram (ERG) parameters of the participants

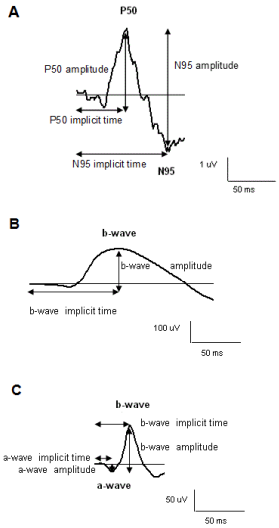
	Cannabis users (n= 53)	Controls (n=29)	*p*-value
Pattern Electroretinogram (PERG)			
N95 Implicit Time (ms) [a, b]	95.5 (91.8:99.9)	88.9 (84.5:91.1)	*p=0.0001*
N95 amplitude (µV) [a,b]	-3.8 (-4.7:-3.3)	-3.7 (-4.6:-3.0)	*p=0.525*
P50 Implicit Time (ms) [a,b]	50.0 (48.4:53.1)	48.6 (47.1:50.8)	*p=0.069*
P50 Amplitude (µV) [a,b]	2.6 (2.2:3.0)	2.3 (2.1:2.7)	*p=0.141*
Flash Electroretinogram (fERG)			
Dark-adapted 0.01 ERG			
b-wave Implicit Time (ms) [a,b]	82.2 (78.7:85.2)[c]	80.9 (77.8:84.6)	*p=0.292*
b-wave amplitude (µV) [a,b]	126.5 (112.8:146.0)[c]	133.0 (120.2:158.7)	*p=0.188*
Light-adapted 3.0 ERG			
a-wave Implicit Time (ms) [a,b]	18.6 (18.6:19.0)	18.6 (18.1:19.0)	*p=0.080*
a-wave amplitude (µV) [a,b]	-10.2 (-11.7:-8.8)	-10.8 (-12.6:-9.2)	*p=0.216*
b-wave Implicit Time (ms) [a,b]	36.3 (35.8:37.2)	35.8 (35.1:36.3)	*p*=0.002
b-wave amplitude (µV) [a,b]	45.4 (40.7:51.2)	48.0 (39.4:51.9)	*p=0.767*

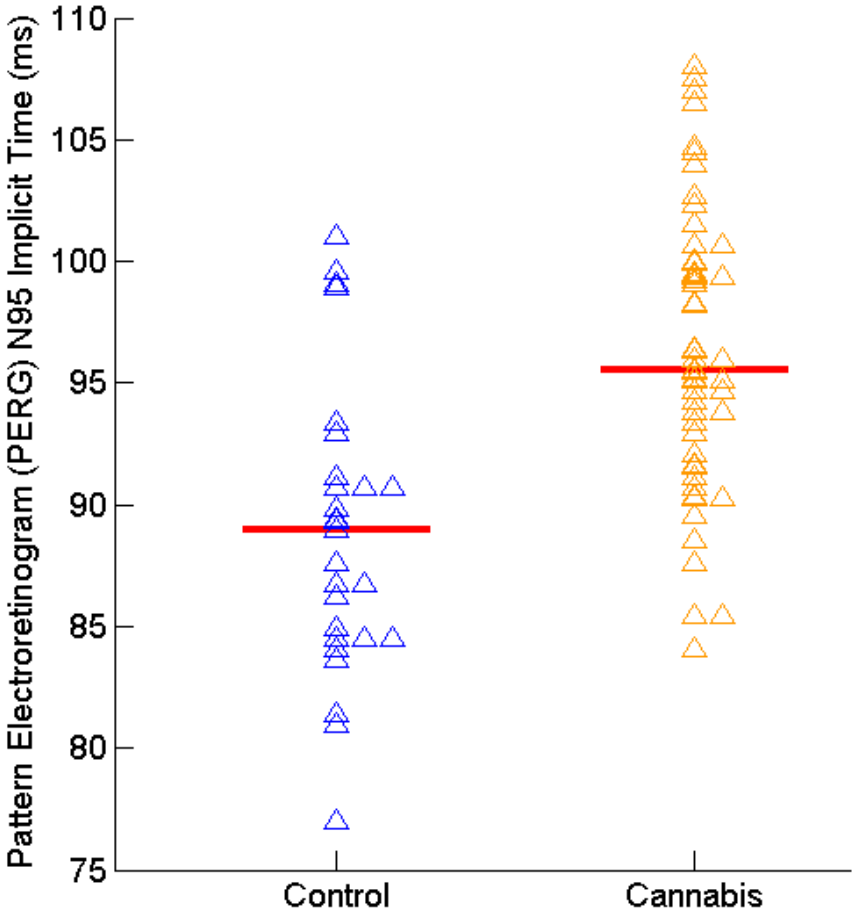
Quantitative variable represented as median and interquartile range [a]
Mann-Whitney U test [b]
n= 52 [c]


Pattern Electroretinogram (PERG) N95 Implicit Time (ms)
110,0
100,0
90,0
80,0
70,0
0
2
4
6
8
10
12
AUDIT Score
Control
Cannabis
Control
Cannabis


Light-adapted 3.0 Electroretinogram (ERG) b-wave Implicit Time (ms)
39,00
38,00
37,00
36,00
35,00
34,00
0
2
4
6
8
10
12
AUDIT Score
Control
Cannabis
Control
Cannabis






Pattern Electroretinogram (PERG) N95 Implicit Time (ms)
110
105
100
95
90
85
80
75
Control
Cannabis