Journal of Psychiatric Research 136 (2021) 312–318

Contents lists available at ScienceDirect

Journal of Psychiatric Research

journal homepage: www.elsevier.com/locate/jpsychires

Delayed on- and off-retinal responses of cones pathways in regular cannabis users: An On-Off flash electroretinogram case-control study

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ARTICLE INFO

Keywords:
Cannabis
Retina
Photopic on-off electroretinogram
Cannabinoid system
Synaptic transmission

ABSTRACT

The retina is considered a useful area for investigating synaptic transmission abnormalities in neuropsychiatric disorders, including as a result of using cannabis, the most widely consumed illicit substance in the developed world.

The impact of regular cannabis use on retinal function has already been evaluated, using pattern and flash electroretinogram (ERG) to demonstrate a delay in ganglion and bipolar cell response. Using multifocal ERG, it was showed that the delay to be preferentially located in the central retina. ERG tests do not separately examine the impact of cannabis on the On and Off pathways. The purpose of this study is to assess On and Off pathway function using On-Off ERG.

We conducted an On-Off ERG test in 42 regular cannabis users and 26 healthy controls. The protocol was compliant with the International Society for Clinical Electrophysiology of Vision (ISCEV) standards. Amplitude and peak time were measured for the a-, b- and d-waves.

Results in the regular cannabis users showed a significant increase in the latencies of both the b- and the d-wave ($p = 0.020$, $p = 0.022$, respectively, Mann-Whitney *U* test), with no change in the wave amplitudes. A-wave peak time and amplitude were unchanged.

These findings are reflective of an effect of regular cannabis use on the On and Off pathways and are consistent with previous findings which also identified increases in retinal neuron response times. We confirm here that regular cannabis use impacts the post-receptoral cones pathway at the level of bipolar cells, affecting the On and Off pathways.

1. Introduction

As an anatomical and developmental extension of the central nervous system (CNS) (Sinn and Wittbrodt, 2013), the retina currently has a crucial role in the neuroscientific investigation of pathophysiological processes (Cosker et al., 2020; Lavoie et al., 2014; London et al., 2013; Schwitzer et al., 2015a; Silverstein and Rosen, 2015). It is a complex neural tissue structure which forms in the embryo from neuroectodermal tissue derived from the diencephalon (Sinn and Wittbrodt, 2013) and is composed of layers of retinal neurons with similar anatomical and functional properties to cerebral neurons (Hoon et al., 2014). These retinal neurons define the initial stages of visual information processing. The primary retinal neurons are the photoreceptors — cones and rods — where photopigments absorb light. The next layers

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https://doi.org/10.1016/j.jpsychires.2021.02.033
Received 11 December 2020; Received in revised form 11 February 2021; Accepted 12 February 2021
Available online 16 February 2021
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are the bipolar cells and the ganglion cells. The axons of the ganglion cells form the optic nerve, which is continuous with the retina at one end and the visual structures of the brain at the other. Retinal neural tissue also contains horizontal and amacrine cells, which function as interneurons (Hoon et al., 2014).

Regular cannabis use is a major public health problem (Degenhardt et al., 2008). Specifically, it is linked to considerable deterioration in cognitive functions — i.e., memory, attention, executive functions or speed of information processing — but also in higher-order brain functions such as intelligence, measured using intelligence quotient (IQ) (Broyd et al., 2016; Meier et al., 2012). Evidence is now beginning to emerge on how cannabis causes these impairments by affecting the structures of the CNS (Bossong and Niesink, 2010; Schwitzer et al., 2015b; Yazulla, 2008). The retina is easy to access, its function is now relatively well understood and we have standardised techniques for exploring its function, ensuring that results are reproducible (Holder et al., 2010). It could therefore provide the means for accurate investigation of the causative mechanisms of such impairments. The widespread distribution of endocannabinoids in the retina, along with their role in regulating physiological functions such as neurotransmission, neuroplasticity and neuroprotection, creates an opportunity to investigate retinal function in regular cannabis users (Schwitzer et al, 2015b, 2016, 2019). The tetrahydrocannabinol (THC) in cannabis joints disrupts the regulatory role played by endocannabinoids.

Previously, we described an effect of regular cannabis use on retinal function (Lucas et al., 2018; Polli et al., 2020; Schwitzer et al, 2017, 2018, 2020), demonstrating a delay of around 6 ms in ganglion cell response as evidenced by increased N95 wave peak time in the pattern ERG in regular cannabis users compared with the control group (Schwitzer et al, 2017, 2018). We also identified a delay of around 0.5–1 ms in cone bipolar cell response, as evidenced by an increase in b-wave peak time in the photopic flash 3.0 ER G (Schwitzer et al., 2018). Lastly, using multifocal ERG we showed increases in peak time in several rings detected on a mfERG trace, such as described here: +1–2 ms for N2 (<2°), N2 and P1 (2–5°), P1 and N1 (5–10°) and P1 (10–15°) (Schwitzer et al., 2020).

The cone system is complex and there are two pathways for transmitting information depending on cone type. L and M cones transmit their information to the On and Off pathways, while S cones transmit their information to a single On pathway type specific to this type of cone (Hoon et al., 2014). The On- and Off-pathways are driven by glutamate receptors (GluRs), especially metabotropic GluRs (mGluRs) and ionotropic GluRs (iGluRs) subtypes. iGluRs are ligand-gated ion channels that produce excitatory glutamate-evoked currents. mGluRs are G protein-coupled receptors (GPCRs) that control cellular processes via G protein signaling cascades (Reiner and Levitz, 2018). These receptors could be involved in the effect of regular cannabis use on retinal function through modulation of glutamatergic pathway.

The pathways can be investigated using On-Off ERG, which measures the function of the photopic On and Off pathways, the entire surface of the retina and its first two layers, comprising the cones and the bipolar cells (Sustar et al., 2018). The On-response appears after the stimuli onset and is characterized by the a- and b-wave. The Off-response appears in response to stimulus offset and consists in d-wave. The principle of the On-Off pathways isolation is the fact that On-bipolar cells depolarize as the response to stimulus onset, while the Off-bipolar cells depolarize as a response to stimulus offset. Thus, the On-Off ERG aims to isolate On-pathway responses from Off-pathway responses (Sustar et al., 2018).

Interestingly, two methods exist to characterise the On- and Off-pathway contribution to the flash ERG. Hamilton et al. used mathematical modelling to impute the On- and Off-pathway contributions using single flashes and applying the model to the plot of the photopic hill (Hamilton et al., 2007). More recently, Gauvin's group has described the DWT (discrete wavelet transform) method that can reveal the relative On- and Off-pathway contributions to the a- and b-waves and the oscillatory potentials (Gauvin et al, 2015, 2017). This method is referenced as an alternative to the extended flash protocol.

Our previous findings identified dysfunctions at the level of b-wave generators, mostly cone-bipolar cells and other post-photoreceptoral strcutures. This paper will therefore focus specifically on the On and Off pathways, as the effect of regular cannabis use on these pathways has not yet been explained. In light of the involvement of the cannabinoid system in cone pathway function (Schwitzer et al, 2015b, 2016, 2019), it can be assumed that the On and Off pathways may both be impacted by regular cannabis use.

2. Material and methods

2.1. Population and ethics statement

Regular cannabis users (n = 42) and age- and sex-matched healthy drug-naïve controls (n = 26) were recruited from 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 describing all aspects of the research. All participants received compensation 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 (NCT02864680, ethical agreement number 13 02 02), which is researching the impact of regular cannabis use on the visual system.

2.2. Inclusion criteria and clinical and biological assessments

The inclusion criteria for the cannabis group was regular cannabis consumption, equating to use on an average of at least 7 occasions per week over the past month. Other 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 cannabis and tobacco are regularly combined 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 h to avoid acute cognitive dysfunction caused by cannabis use.

The inclusion criterion for healthy controls were no history of illicit substance use, a negative urine toxicology screen for THC metabolites and other illicit drugs tested and no history of DSM-IV diagnosis of Axis I psychiatric disorders. All participants were aged 18–35 years, had no history of neurological disease, no family history of schizophrenia or bipolar disorders, and were medication-free except for women with oral contraceptives. They had no history of ophthalmological disease except for corrected refractive errors. All of them performed 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. Participants reporting alcohol dependence based on their score in the Alcohol Use Disorders Identification Test (AUDIT) were excluded from the study. The Mini-International Neuropsychiatric Interview (M.I.N.I.) was administered to assess current and past history of psychiatric diseases and substance use. In addition, the Cannabis Abuse Screening Test (CAST) (score: 0-<3: use, no addiction risk; 3-<7: low addiction risk; >7: high addiction risk) (Legleye et al., 2015), Fagerström test (score: 0–2: no dependence; 3–4: slight dependence: 5–6: middle dependence; 7–10: high dependence) (Heatherton et al., 1991) and the AUDIT (score: ≥5: risky use; ≥8: harmful use: ≥12: dependence) (Saunders et al., 1993) were performed to assess use, abuse and dependence with respect to cannabis, tobacco and alcohol respectively.

The extent of cannabis use was assessed clinically by means of an interview and a questionnaire to elicit the following information: age when regular cannabis use began, total number of years of cannabis use, average number of joints smoked per day and per week over the past month and average number of grams smoked per week (Table 1). Urine drug screens (nal von minden, Moers, Germany) were immediately performed prior to electroretinogram testing to confirm cannabis consumption and to eliminate use of buprenorphine, benzodiazepines, cocaine, opiates, amphetamines and methadone.

2.3. Experimental protocol

Photopic On-Off ERG was performed according to the International Society for Clinical Electrophysiology of Vision (ISCEV) standards for this test (Sustar et al., 2018). The MonPackONE system (Metrovision, Pérenchies, France) was used for stimulation, recording and analysis. The equipment used met the ISCEV full-field standard. Electrical signals from both eyes were recorded simultaneously. First, averaged retinal responses were obtained from each eye and values for the peak time and amplitude parameters were then averaged across both eyes for analysis. Electrical signals were recorded with dilated pupils (Tropicamide 0.5%), using DTL electrodes (Metrovision, Pérenchies, France) placed in the bottom of the conjunctival sac. Pupil size was noted before and after photopic On-Off ERG recordings and remained systematically constant throughout the testing period. The pupil sizes of both groups (cannabis users/controls) were the same. Ground and reference electrodes were attached to the forehead and external canthi.

2.4. Photopic On-Off ERG measurements

Photopic On-Off ERG recordings were performed in light-adapted conditions, projecting a white stimulus onto a white background for a duration of 150 ms. The luminance of the stimulus was 250 cd/m^2, while the background luminance was 30 cd/m^2. Stimuli were delivered at a rate of two per second. Participants were placed at a distance of 30 cm from the screen. They were light-adapted for a period of 10 min before photopic On-Off ERG was performed. At least 16 responses were recorded for each participant.

Table 1
Demographic and substance use characteristics of the participants.

	Cannabis users (n = 42)	Controls (n = 26)	*P*-value
Gender (male/female) [a,d]	34/8	21/5	*p = 0.99*
Age (years) [b,c]	23 (20–29.25)	24 (22.75–28.25)	*p = 0.167*
Education (years) [b,c]	13 (12–14.25)	15 (14–16)	*p = 0.0001*
Average number of alcohol uses/week [b,c]	4.50 (2–10)	1 (0–3)	*p = 0.0001*
Alcohol Use Disorders Identification Test (AUDIT) scores [b,c]	7 (4–11)	2.5 (1–4)	*p = 0.0001*
Fagerström Test scores [b]	1 (0–3.25)	–	–
Average number of cigarettes/day [b]	5 (2–10)	–	–
Age of first cannabis use [b]	16 (14.75–16)	–	–
Total years of cannabis use [b]	7 (5–13.25)	–	–
Average number of joints/week [b]	20 (13–25)	–	–
Cannabis Abuse Screening Test (CAST) scores [b]	4 (3–5)	–	–
Average number of grams of cannabis/week [b]	4 (2.88–8.5)	–	–

[a] Categorical variable represented as frequencies.
[b] Quantitative variable represented as median and interquartile range.
[c] *Mann-Whitney U test.*
[d] *Test exact de Fisher.*

2.5. Analysis

Photopic On-Off ERG data were analysed using the ophthalmic monitor MonPackOne (Metrovision, Pérenchies, France). Analysis was performed with the investigator blind to the status of the subject being recorded (cannabis user or control). Two main responses are usually described on a typical photopic On-Off ERG trace: the On-response and the Off-response. The On-response occurs after stimulus onset and is characterized by two waves: a negative a-wave and a positive b-wave. The Off-response d-wave is a positive wave evoked by stimulus offset. Two main parameters are derived from the a-wave, b-wave and d-wave and are conventionally known as amplitude, measured in microvolts (μV), and peak time, measured in milliseconds (ms). A-wave amplitude is measured from the baseline to the negative trough of the a-wave. B-wave amplitude is measured from the trough of the a-wave to the peak of the b-wave. D-wave amplitude is measured from the time point of stimulus offset to the peak of the d-wave. On-response peak times are measured from the beginning of stimulus to the trough of the a-wave and the peak of the b-wave; Off-response peak time is measured from the beginning of stimulus to the peak of the d-wave (Sustar et al., 2018). Fig. 1 shows a typical trace for the three main components of the photopic On-Off ERG and their measurement.

2.6. Statistical analysis

Based on the non-parametric distribution of continuous variables included in the analyses, a Mann-Whitney *U* test, Chi-square test and Spearman rank correlation coefficient were used as appropriate for comparison or relationship analysis within the two cannabis user/control groups. Categorical variables were shown as numbers and quantitative variables were given as medians and interquartile ranges (IQR). An alpha level of 0.05 was used and statistical analyses were performed using IBM-SPSS Statistics 25.0 (IBM Corp.).

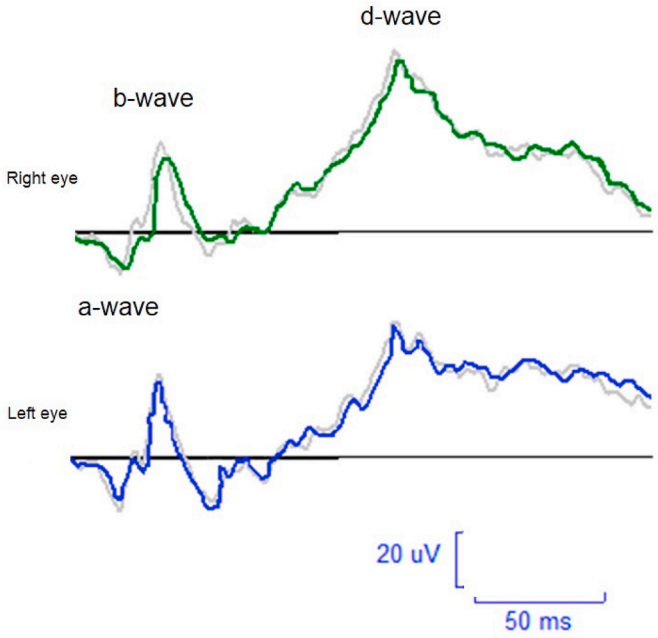

Fig. 1. Typical photopic On-Off ERG traces for right and left eye with a-, b- and d-wave. Two traces of cannabis users are superimposed to demonstrate reproducibility.

3. Results

3.1. 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 = 0.167) or gender (p = 0.99), 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 = 0.0001 for average alcohol consumption/week; p = 0.0001 for AUDIT score). Because tobacco is widely mixed with cannabis in joints, all cannabis users were also tobacco smokers, whereas all the controls were non-smokers. According to the Fagerström test, 28 in 42 cannabis users were not dependent on nicotine, 10 were slightly dependent, 4 were mildly dependent and no one was highly dependent.

3.2. Photopic On-Off ERG responses

The photopic On-Off ERG responses parameters are described in Table 2.

Photopic On- ERG response: The median and interquartile range of the a-wave peak time was 20.40 ms (19.50: 20.95) in cannabis users versus 20.15 ms (19.05: 20.85) in controls. This difference was not significant between groups (p = 0.172*;* Mann-Whitney *U* test). The median and interquartile range of the a-wave amplitude was −10.68 μV (−14.81: 6,94) in cannabis users versus −10.90 μV (−15.74: 9.25) in controls. This difference was not significant between groups (p = 0.672*;* Mann-Whitney *U* test). The median and interquartile range of the b-wave peak time was 34.95 ms (34.50: 36.75) in cannabis users versus 34.28 ms (33.60: 34.95) in controls. This difference was significant between groups (p = 0.020*;* Mann-Whitney *U* test) (Fig. 2A). The median and interquartile range of the b-wave amplitude was 26.80 μV (21.46: 31.56) in cannabis users versus 28.70 μV (23.23: 33.89) in controls. This difference was not significant between groups (p = 0.165*;* Mann-Whitney *U* test).

Photopic Off- ERG response: The median and interquartile range of the d-wave peak time was 127.50 ms (127.00: 128.50) in cannabis users versus 127.00 ms (126.50: 127.63) in controls. This difference was significant between groups (p = 0.022*;* Mann-Whitney *U* test) (Fig. 2B). The median and interquartile range of the d-wave amplitude was 13.15 μV (2.70: 18.98) in cannabis users versus 9.35 μV (1.84: 19.84) in controls. This difference was not significant between groups (p = 0.686*;* Mann-Whitney *U* test).

Spearman rank order correlations showed no significant correlations between the number of cigarettes per day or the Fagerström scores with ERG measures in the cannabis users group.

Table 2
Electroretinogram (ERG) photopic On-Off parameters of the participants.

	Cannabis users (n = 42)	Controls (n = 26)	*p*-value
a-wave peak time (ms)[a,b]	20,40 (19.50:20.95)	20.15 (19.05:20.85)	*p = 0.172*
a-wave amplitude (μV)[a,b]	−10.68 (−14.81: 6.94)	−10.90 (−15.74: 9.25)	*p = 0.672*
b-wave peak time (ms)[a,b]	34.95 (34.50:36.75)	34.28 (33.60:34.95)	*p = 0.020*
b-wave amplitude (μV)[a,b]	26.80 (21.46:31.56)	28.70 (23.23:33.89)	*p = 0.165*
d-wave peak time (ms)[a,b]	127.50 (127.00:128.50)	127.00 (126.50:127.63)	*p = 0.022*
d-wave amplitude (μV)[a,b]	13.15 (2.70:18.98)	9.35 (1.84:19.84)	*p = 0.686*

[a] Quantitative variable represented as median and interquartile range.
[b] Mann-Whitney *U* test.

4. Discussion

We have shown here that the On and Off pathways were both impaired by regular cannabis consumption. Previous studies had been unable to isolate the pathways to draw conclusions on the effect of cannabis on each pathway. In our study, there is a significant increase in b-wave and d-wave peak time in the cannabis users' group compared with the control group. These findings are consistent with and confirm our earlier results as we again see an increase in wave peak time, reflecting a delay in cellular response. Investigation of the On and Off cone pathways makes it clear that regular cannabis use does indeed alter the whole of the cone system, affecting specific characteristics of each of its pathways. We observed no difference in the kinetics of the a-wave although the a-wave of the photopic ERG is also shaped by the Off-pathway (Bush and Sieving, 1994). However, we suppose that the participation of the Off-pathway is insufficient to induce modulation of the a-wave peak time. This is consistent with our previous results which also show no difference in the a-wave peak time of the photopic 3.0 fERG (Schwitzer et al., 2018).

These findings are reflective of an effect of the THC in smoked cannabis which disrupts the regulatory role of the retinal cannabinoid system. The cannabinoid system comprises the cannabinoid CB1 and CB2 receptors, their ligands — primarily N-arachidonoylethanolamine (anandamide or AEA) and 2-arachidonoylglycerol (2-AG) — and the enzymes that regulate them. CB1 receptor expression in humans is found in the various layers of the retina (Porcella et al., 2000; Straiker et al., 1999; Wei et al., 2009). The current scientific data make it difficult to differentiate between the selective expression of CB1 and CB2 receptors on the On or Off cone pathways. The CB1 and CB2 receptors are stimulated primarily by two endogenous ligands: 2-AG, which is present in the human retina at significant concentrations, and anandamide, which is present at lower concentrations (Chen et al., 2005; Devane et al., 1992; Mechoulam and Parker, 2013; Stamer et al., 2001). The most important enzymes in regulating the concentration of these ligands are diacylglycerol lipase alpha and beta (DAGLα and DAGLβ) (Bisogno et al., 2003), N-acyl-phosphatidylethanolamine-phospholipase D (NAPE-PLD) (Di Marzo et al., 1994; Pertwee et al., 2010), cyclooxygenase 2 (COX-2) (Yazulla, 2008), fatty acid amide hydrolase (FAAH) (Pertwee, 2005) and monoacylglycerol lipase (MGL) (Pertwee, 2005). There is currently little accurate information about the presence of these ligands and enzymes in the retina, particularly on the On and Off pathways. Some scientific research has nonetheless identified diacylglycerol lipase alpha (DAGLα) in the postsynaptic terminals of cone Off bipolar cells in the outer and inner plexiform retinal layers in mice (Hu et al., 2010).

In conjunction with ion channels, CB1 receptors modulate the release in the retina of neurotransmitters such as glutamate, gamma-Aminobutyric acid (GABA) and dopamine. These neurotransmitters play a major role in processing visual information in the retina and in the vertical transmission of visual signals (Schwitzer et al., 2015b, 2016, 2019; Yazulla, 2008). Some studies have in fact identified the presence of glutamate receptors in the photoreceptors, the bipolar cells and the ganglion cells (Wu and Maple, 1998). GABA receptors are present in the photoreceptors, bipolar cells, ganglion cells and horizontal cells (Lukasiewicz and Shields, 1998). Scientific studies to date neither confirm nor disprove this receptor activity on the cone On or Off pathways. In mammals, D1-class dopamine receptors (D1-R) are present in the ganglion, bipolar and horizontal cells while D2-class receptors (C2-R) are found in the photoreceptors, bipolar cells and horizontal cells (Nguyen-Legros et al., 1997). The findings of our study show that visual transmission along the On and Off cone pathways is delayed in regular cannabis smokers, suggesting that the cannabinoid system may be present on each of these pathways. Additional investigations demonstrating that the cannabinoid system is indeed present on both the On and Off cone pathways in humans would confirm this hypothesis.

Taken together, our results in regular cannabis users showed: 1- an increase of ~ 6 ms in N95 peak time of the PERG; 2- an increase of ~ 1

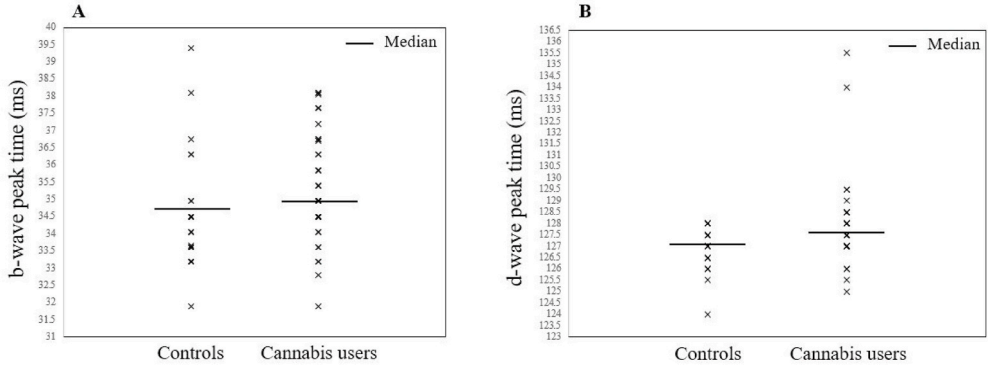

Fig. 2. A. Dot plot On-Off electroretinogram b-wave peak time for cannabis users (n = 42) and controls (n = 26) with medians. Cannabis users showed increased peak time and the difference between the groups is significant (p = 0.020). **B.** Dot plot On-Off electroretinogram d-wave peak time for cannabis users (n = 42) and controls (n = 26) with medians. Cannabis users showed increased peak time and the difference between the groups is significant (p = 0.022).

ms in b-wave peak time in photopic condition of the fERG; 3- increases in peak time of ~ +1–2 ms for N2 (<2°), N2 and P1 (2–5°), P1 and N1 (5–10°) and P1 (10–15°) of the mfERG; 4- decrease in OP2 and OP3 amplitudes of ~5 and 7 μV; 5- increases in b- and d-wave peak time of the photopic On-Off ERG. Overall, these results suggest alterations of the ganglion cells, cone system and amacrine cells functions. Regular cannabis use is associated with decrease in the speed of information processing in the CNS (Broyd et al., 2016; Meier et al., 2012). Our results based on delayed retinal responses support that information processing is processed less rapidly in regular users. Importantly, these results are consistent since delayed responses -as observed by increases in peak time-were found at several retinal levels. Retinal processing is slowed down at several cellular levels and in several pathways -On and Off-. Interestingly, increased peak times were found with both fERG and PERG -giving a true physiological response- and with mfERG -a mathematical extraction of the physiological retinal responses-. This supports the consistency of the results. Cannabis is known to be a neuromodulator substance acting on several neurotransmission pathways in the CNS, especially glutamatergic, gabaergic and dopaminergic pathways (Bossong and Niesink, 2010; Colizzi et al., 2016; Sami et al., 2015). We suppose that the delayed retinal responses observed in regular users are the consequence of the impact of cannabis on several neurotransmitters such as glutamate, GABA and dopamine, in accordance with the effects of regular cannabis use on the brain. Since the retina is part of the CNS due to its embryonic origin, these results may be viewed as a reflect of the cerebral impact of regular cannabis use. There appears to be a difference in the timing of the retinal signaling pathways in light-adapted and dark-adapted conditions. These results may also be the consequence of regular cannabis use on the kinetics of ion channels. Cannabinoid agonists induce a dose-dependent reversible modulation of calcium, potassium and chloride currents in bipolar, rod, cone and ganglion cells (Schwitzer et al, 2015b, 2016). These modulations could be at the origin of the retinal dysfunctions observed in regular users. Future studies should evaluate pathophysiological mechanisms at this level by assessing the potential modulation of ionic currents such as calcium, potassium, sodium and chloride currents at the level of bipolar, cone, amacrine and ganglion cells, to name a few. The fact that amplitudes of OPs are decreased whereas peak times of N95, b-, d-, N1, P1 and N2 waves are increased may be linked to the characteristics of OPs. Indeed, OPs are similar to a pseudo periodic and sinusoidal signal with small changes in peak time but with a potentially higher impact on the amplitude of OPs waves. In our study, peak times of OPs are highly reproducible between participants which may probably be due to the characteristics of this sequence.

This study opens up a number of possibilities. Indeed, THC disrupts glutamate neurotransmission and the role of THC in potentiating the risk of conversion to psychosis is well documented (Di Forti et al., 2019). This entanglement is even more decisive regarding the glutamate hypothesis of schizophrenia (Kantrowitz and Javitt, 2012) and emerging evidence of potential similar glutamatergic retinal dysfunctions in schizophrenia (Bernardin et al., 2020). Hence it appears crucial to further explore at the retinal level neurotransmission abnormalities induced by THC in smokers with no history of psychosis. This could provide additional knowledge for elaborating a neurotransmission model for THC in increasing the risk of developing psychosis or the deleterious impact for prognosis of cannabis consumption in patient with psychosis (Manrique-Garcia et al., 2014).

One study identified a change in b-wave and d-wave peak time in the On-Off ERG in response to the human nycthemeral cycle (Hankins et al., 2001), with the authors finding that b-wave and d-wave peak time was reduced during the day compared with night-time. The shortest latencies were seen at around midday during daylight hours over a 24-h cycle. This suggests that the diurnal effect probably resides in the cone synapses. There is a similar difference in b-wave and d-wave latencies in regular cannabis smokers compared with healthy non-smokers and between daytime and night-time states in a single individual. This raises the question of whether long-term cannabis use results in a change in the nycthemeral cycle, given that cannabis is known to disrupt sleep patterns and can reverse the circadian rhythm. Only few studies have addressed the On and Off pathways using On-Off ERG in patient populations with neuropsychiatric disorders. One study showed selective impairment of the On pathway in a group of patients with autistic spectrum disorder (ASD) compared with a control group (Constable et al., 2016a). The authors suggest that the cone On-bipolar cell signaling pathway is impaired in this pathology. This study was then expanded and showed that the ASD group had smaller b- and a-wave amplitudes at high flash strengths and slower b-wave peak times (Constable et al., 2020).

We know that regular cannabis, alcohol or tobacco consumption affects the release of certain neurotransmitters (Bossong and Niesink, 2010; Schwitzer et al., 2019). In addition, cannabis, tobacco and alcohol are psychoactive substances with neuromodulating effects that can be potentiated (Lucas et al., 2018). Most of the cannabis users in our study also smoke and drink alcohol. Separate investigation of the effect of each these substances on retinal function and On-Off ERG with discrete groups of regular alcohol, tobacco and cannabis users is therefore essential, despite the fact that it has been demonstrated statistically that the findings of prior studies were not dependent on tobacco or alcohol use. To our knowledge, no studies have investigated the effect of regular use of tobacco and alcohol on the On-Off ERG. In this study, the time between last joint and ERG recording was not recorded and included in the analyses. Withdrawal symptoms were not evaluated with specific scales. Since they may influence the results, further studies should consider these parameters. The abnormalities described here are functional impairments with underlying pathophysiological mechanisms that have not yet been objectively identified. Future genetic and

molecular studies could be valuable for discovering which specific cell signaling pathways are affected in cannabis smokers. Regular cannabis smokers have been observed to have an impaired quality of life (Aspis et al., 2015). It would be useful to investigate whether the On-Off ERG parameters, such as b-wave and d-wave peak time, correspond to the changes in quality of life, and could potentially help to predict quality of life in cannabis smokers. Finally, the concentration of THC and cannabidiol (CBD) was not assessed in this study. These measures should be added in future studies in the field to increase knowledge on the effect of the two major exocannabinoid ligands on retinal processing.

Author contributions

All the authors contributed to write the manuscript, concurred with the submission and have approved the final manuscript.

Funding

This study was supported by a French National Research Agency (ANR) Grant (ANR-12-SAMA-0016-01) and by the French Mission Interministérielle de Lutte Contre les Drogues et les Conduites Addictives (MILDECA). ANR and MILDECA have no role in design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Ethical statement

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.

Declaration of competing interest

All the authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgements

The authors thank the Ophtalmology staff of Nancy University Hospital and MetroVision for their contribution to this work. The authors also thank all members of the CAUSA MAP study group: Marc Borie, CHRU Nancy, France; Stéphanie Caharel, InterPsy, Université Lorraine, France; Paolo Di Patrizio, Université Lorraine, France; Philip Gorwood, Centre de Psychiatrie et Neurosciences, Paris, France; Coline Jeantet, InterPsy, Université Lorraine, France; Julien Krieg, INSERM U1114, Université Strasbourg; Joëlle Lighezzolo-Alnot, InterPsy, Université Lorraine, France; Valérie Louis Dorr, CRAN CNRS UMR 7039, Nancy, France; Louis Maillard, CRAN CNRS UMR 7039, Nancy, France; Nicolas Ramoz, Centre de Psychiatrie et Neurosciences, Paris, France.

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