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Spatial localization of retinal anomalies in regular cannabis users: The relevance of the multifocal electroretinogram

Thomas Schwitzer [a,b,*], Marie-Laure Henrion [a,1], Daphné Sarre [a,1], Eliane Albuisson [e,f,g], Karine Angioi-Duprez [d], Anne Giersch [b], Laurence Lalanne [h], Raymund Schwan [a,b,c], Vincent Laprevote [a,b]

[a] Pôle Hospitalo-Universitaire de Psychiatrie d'Adultes du Grand Nancy, Centre Psychothérapique de Nancy, Laxou, France
[b] INSERM U1114, Fédération de Médecine Translationnelle de Strasbourg, Département de Psychiatrie, Centre Hospitalier Régional Universitaire de Strasbourg, Strasbourg, France
[c] Maison des Addictions, CHRU Nancy, Nancy, France
[d] Service d'Ophtalmologie, CHRU Nancy, Nancy, France
[e] Pôle S^2R, PARC, BIOBASE, CHRU Nancy, Vandoeuvre lès Nancy, France
[f] Université de Lorraine, Faculté de Médecine, InSciDens, Vandoeuvre lès Nancy, France
[g] CNRS, Institut Elie Cartan de Lorraine, UMR 7502, Vandoeuvre-lès-Nancy F-54506,France
[h] INSERM 1114, Department of addictology, FMTS (federation de médecine translationnelle) University Hospital of Strasbourg

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ABSTRACT

Widely used in industrialized countries, cannabis is a neuromodulator substance. The cannabinoid system is present at critical stages of retinal processing. We have recently shown a delay in bipolar and ganglion cell responses in regular cannabis users, as observed using flash (fERG) and pattern (PERG) electroretinogram. Although the results obtained during these tests provide information about global retinal responses, they do not give any indication about the spatial localization of the anomalies that were detected. The latter may be analyzed, however, by means of multifocal electroretinogram (mfERG). We recorded the mfERG responses in 49 regular cannabis users and 21 healthy subjects. The amplitudes and implicit times of the mfERG N1, P1 and N2 waves were recorded. The results showed that in regular cannabis users: in the <2° region, a significant increase in the N2 implicit time ($p = 0.037$); in the 2–5° region, a significant increase in the N2 ($p = 0.018$) and P1 ($p = 0.046$) implicit times; in the 5–10° region, a significant increase in the P1 ($p = 0.006$) and N1 ($p = 0.034$) implicit times; and in the 10–15° region, a significant increase in the P1 implicit time ($p = 0.014$). An isolated decrease in the N1 amplitude in the 2–5° region ($p = 0.044$) was also found. This indicates that there is a delay in the transmission of visual information from the central retina to the near periphery in cannabis users suggesting potential alterations in precise and color vision.

1. Introduction

Cannabis use is an increasing public health challenge since it is one of the most psychoactive drugs used worldwide (Degenhardt et al., 2008). Additionally, cannabis is known to be a neuromodulator substance that acts on several neurotransmission pathways such as glutamatergic and dopaminergic pathways (Bossong and Niesink, 2010; Schwitzer et al., 2015; Yazulla, 2008).

Our group recently demonstrated that there is a delay in the transmission of visual information from the retina to the brain in regular cannabis users. This delay was found at two retinal levels: ≅1 millisecond (ms) for bipolar cells and measured with flash electroretinogram (fERG) – b-wave of photopic 3.0 ERG – and ≅6 ms for ganglion cells observed with pattern ERG (PERG) – N95 wave – (Schwitzer et al., 2017, 2018). In other words, the visual signal leaving the retina to travel to the brain via the optic nerve is delayed by approximatively 6 ms in regular cannabis users. These retinal dysfunctions have been identified as being a potential consequence of cannabis use on retinal neurotransmission, especially glutamatergic retinal transmission, since glutamate is a key neurotransmitter involved in the vertical transmission of retinal information (de Souza et al., 2013).

These findings, as well as demonstrating the impact of cannabis use on retinal function, could also help us understand the effects of cannabis on visual perception. Indeed, several case reports and case series have underlined the effects of cannabis on vision (Lalanne et al., 2017; Schwitzer et al., 2015). For example, cannabis users reported improved vision, blurred vision, flickering black spots, visual distortions, illusions of movement, color vision disturbances, to name a few. These effects are mediated through an action of exocannabinoids such as

* Corresponding author at: Psychotherapic Center of Nancy, 1, rue du Docteur Archambault, Laxou F-54 521, France.
E-mail address: thomas.schwitzer@univ-lorraine.fr (T. Schwitzer).
[1] Contributed equally to this work.

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tetrahydrocannabinol on the endocannabinoid system (Bossong and Niesink, 2010; Schwitzer et al., 2015). To this date, how these effects are exerted is unknown but we suppose that part of them have a retinal origin. However, the findings were extracted from fERG and PERG examinations that provide information about global responses of the retina (Bach et al., 2013; McCulloch et al., 2015). PERG, which shows the largest delay in cannabis users, comes from the macular region. In order to assess whether the delay observed is from the photoreceptors or bipolar cells, we recorded mfERG in the sample of regular cannabis users from our previous study.

The mfERG records the response of the central retina (Hood, 2000). The stimulus is composed of multiple hexagons gradually increasing in size from the center to the periphery of the screen (Holder et al., 2010). Each hexagon is illuminated pseudo-randomly by a flash stimulation and elicits a local response in the retinal cone system. The mfERG recordings make it possible to evaluate multiple local responses derived from each hexagon. Three main waves are regularly described: N1, P1, and N2. N1 represents the first electronegative component followed by an electropositive wave P1, itself followed by an electronegative component N2. Both the amplitudes and implicit times of each wave were evaluated (Hood et al., 2012) (Fig. 1). N1 for the most part originates from the hyperpolarization of the OFF bipolar cells and P1 for the most part originates from the depolarization of the ON bipolar cells. It is likely that N1 partially shares origin with the fERG a-wave recorded in photopic condition, and the P1 and N2 waves with the fERG b-wave recorded in photopic condition (Holder et al., 2010).

The aim of this study was to evaluate the spatial properties of the retinal cone system by means of mfERG measurements in regular cannabis users compared with controls. Our hypothesis was that retinal dysfunctions previously observed with PERG in regular cannabis users were caused by a specific spatial distribution of alterations of the retinal function.

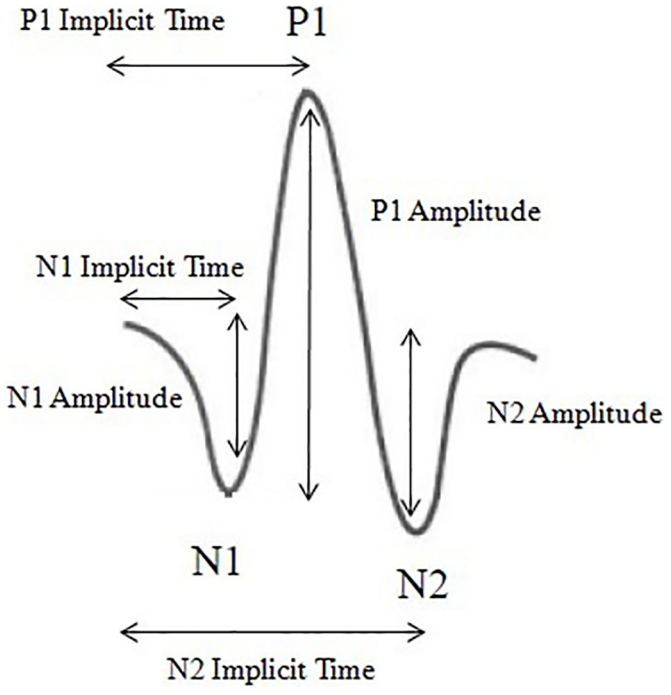

Fig. 1. Typical multifocal ERG (mfERG) wave with the three main components: N1, P1 and N2. Amplitude (uV) and implicit time (ms) are derived from these waves.

2. Material and methods

2.1. Population and ethics statement

Among the 53 regular cannabis users and the 29 healthy drug naive controls recruited in the previous study (Schwitzer et al., 2018), respectively 4 and 8 were excluded from each group (cannabis users and controls) because data were not recorded or not interpretable. Thus, 49 regular cannabis users and 21 matched healthy drug naive controls – who were part of this previous study – were recruited for this study. Modalities of recruitment, inclusion criteria, clinical and biological assessments of each group – cannabis users and controls – have been previously described in Schwitzer et al. (2018). Briefly, volunteers underwent a full psychiatric evaluation. The Mini-International Neuropsychiatric Interview (M.I.N.I.) was used to assess current and past history of psychiatric diseases and substance use. All participants had no DSM-IV diagnosis of Axis I disorders evaluated by MINI. Participants signed consent forms detailing all aspects of the research. All participants received payment of €100. 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.

2.2. Experimental protocol

mfERG was performed in accordance with International Society for Clinical Electrophysiology of Vision (ISCEV) standards (Hood et al., 2012). Stimulation, recording and analysis were performed with the MonPackONE system (Metrovision, France). Using Dawson-Trick-Litzkow (DTL) electrodes placed at the bottom of the conjunctival sac, we recorded electrical signals by monocular stimulations for each eye, on dilated pupils (Tropicamide 0.5%). Pupil's size was systematically noted before and after recordings and remained constant during the whole testing period. Ground and reference electrodes were fixed to the forehead and external canthi.

The stimulus matrix consisted of an array of 61 scaled hexagonal elements corresponding to the central 20°. These were modulated between white and black according to a pseudorandom sequence. The luminance of the stimulation was 100 candela/m^2 (cd/m^2). The stimulus screen was surrounded by a uniformly illuminated background cover with the luminance set at 30 cd/m^2, managed by the MonPackOne system, to eliminate the rod response. The stimuli frequency was set at 75 Hz. Participants were positioned 30 cm from the screen. All of the subjects were fully corrected optically for the viewing distance and asked to fixate the central red target. Any segments associated with blinks or eye movements were rejected. At least 5000 responses were recorded for each eye of each participant with a level of noise maintained under 5 kiloohm (kΩ) to achieve the best signal-to-noise ratio. A control video given by the computer allows one to track the fixation of the participants during the recordings online by giving the position of the eyes. In the case of abnormal fixations, responses were rejected.

MfERG data was analyzed with Monitor Ophthalmic (Metrovision, France). Analysis was performed with the experimenter blind to the status of the subject being recorded (cannabis users or control). MfERG responses were averaged over five retinal regions: <2°, 2–5°, 5–10°, 10–15°, and >15°. Three main components are usually described on a typical mfERG trace: a first negative wave called N1, followed by an electropositive component P1, and then a second negative wave N2. The origin of each wave is described in the introduction. A trace of a multifocal ERG (mfERG) recording (A) and a 3-D map of mfERG showing the central foveal response (B) for the right eye of a cannabis user is represented in Fig. 2.

Two main parameters are derived from N1, P1 and N2, known by convention as the amplitude measured in microvolts (uV) and the implicit time measured in ms. The amplitude of N1 was measured from

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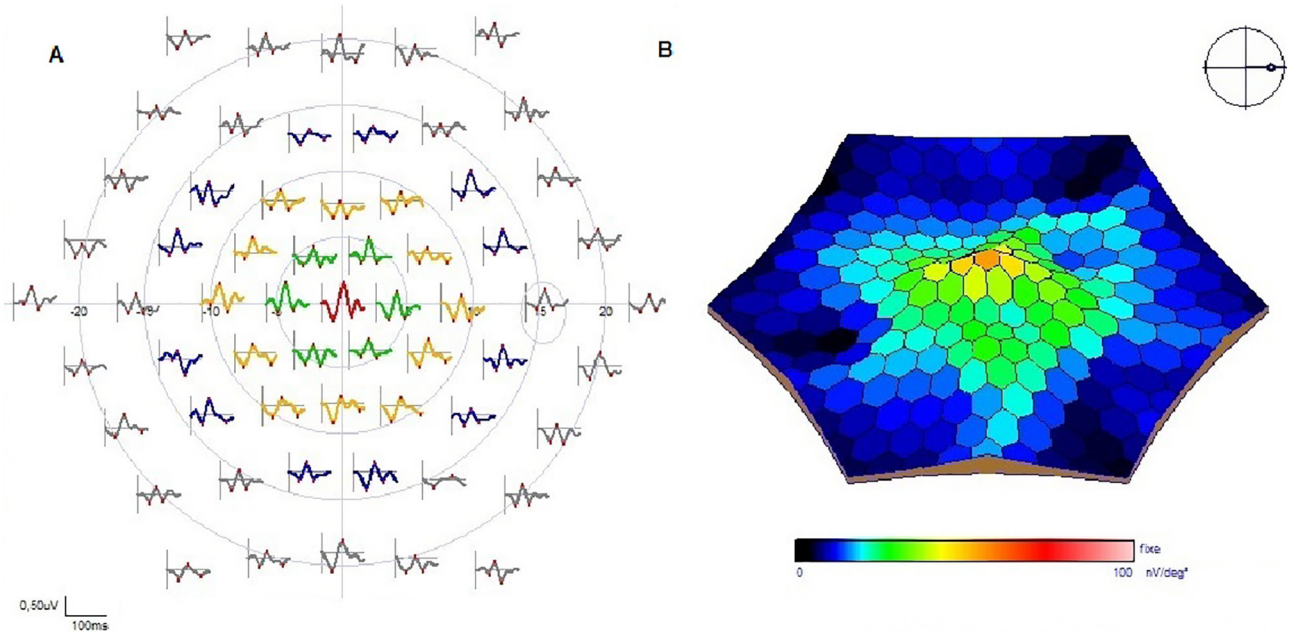

Fig. 2. Multifocal ERG (mfERG) recording (A) and 3-D map of mfERG showing the central foveal response (B) for the right eye of a cannabis user.

the baseline to the trough of N1. The amplitude of P1 and N2 are the trough-to-peak amplitude, measured respectively from the trough of N1 to the peak of P1, and from the peak of P1 to the trough of N2. Implicit time denotes the time taken to reach the maximum N1, P1 and N2 amplitudes. Responses were recorded from each eye. Analysis was done after averaging over both eyes.

2.3. 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 rank correlation coefficient were used when appropriate to analyze comparison or relation within the two cannabis user/control groups. Categorical variables were represented as number and quantitative variables were represented as median and interquartile range (IQR) since the median and IQR are less sensitive to extreme values and less dependent of the distribution of values. We used a level of significance with alpha <0.05. Statistical analyses were performed using IBM-SPSS Statistics 22.0 (IBM corps.).

3. Results

3.1. Demographic and substance use characteristics

Table 1 summarizes the demographic and substance use characteristics of the cannabis users and controls. We noted no relevant difference between groups in terms of age ($p = 0.612$), gender ($p = 0.951$) or average alcohol consumption/week ($p = 0.055$). Differences were observed, however, between groups in terms of years of education ($p = 0.006$; lower in cannabis users) and AUDIT score (higher in cannabis users; $p = 0.004$). 43 in 49 cannabis users were also tobacco smokers. All the controls were non-smokers. Based on the Fagerström test, 24 in 49 cannabis users were not dependent on tobacco; 11 in 49 were slightly dependent; 8 in 49 were mildly dependent; and none of the cannabis users was highly dependent.

3.2. Multifocal electroretinogram (mfERG) parameters

The data for the mfERG measurements of participants – cannabis users and controls – are described in Table 2.

In ring 1 (<2°), the median and IQR of the N2 implicit time was 71.90 ms [70.30: 73.35] in cannabis users versus 70.50 ms [69.20: 72.20] in controls. This difference was significant between groups ($p = 0.037$) (Fig. 3A).

In ring 2 (2–5°), the median and IQR of P1 implicit time was 46.15 ms [45.00: 46.95] in cannabis users versus 45.60 ms [43.90: 46.25] in controls. This difference was significant between groups ($p = 0.046$) (Fig. 3B). The median and IQR of N2 implicit time was

Table 1 t1.1
Demographic and substance use characteristics of the participants. t1.2

	Cannabis users (n = 49)	Controls (n = 21)	p-Value	t1.3
Gender (male/female)[a,d]	37/12	16/5	p = 0,951	t1.4
Age (years)[b,c]	23 [21−30]	24 [23–26]	p = 0,612	t1.5
Education (years)[b,c]	13 [11–14]	15 [14–16]	p = 0,006	t1.6
Average number of alcohol uses/week (in French standard unit)[b,c]	3 [1–7]	1 [0–3,5]	p = 0,055	t1.7 t1.8
Alcohol Use Disorders Identification Test (AUDIT) scores[b,c]	6 [3–9]	2 [1–5]	p = 0,004	t1.9 t1.10
Fagerström Test scores (n = 43)[b]	1 [0–4]	–	–	t1.11
Average number of cigarettes/day[b]	5 [2−10]	–	–	t1.12
Average number of pack-year[b]	2 [1–5]	–	–	t1.13
Age of first cannabis use[b]	16 [15–17]	–	–	t1.14
Total years of cannabis use[b]	7 [5–14]	–	–	t1.15
Average number of joints/week[b]	20 [14–30]	–	–	t1.16
Cannabis Abuse Screening Test (CAST) scores[b]	4 [3–5]	–	–	t1.17
Average number of grams of cannabis/week[b]	4,2 [3−10]	–	–	t1.18

Educational level (years) is evaluated by the number of years of study after the first class of primary school. t1.19 t1.20
AUDIT score was performed to assess use, abuse and dependence with respect to alcohol. t1.21
Fagerström test was performed to assess use, abuse and dependence with respect to tobacco. t1.22 t1.23
Cannabis Abuse Screening Test (CAST) was performed to assess use, abuse and dependence with respect to cannabis. t1.24 t1.25
[a] Categorical variable represented as number. t1.26
[b] Quantitative variable represented as median and interquartile range. t1.27
[c] Mann-Whitney *U* test. t1.28
[d] Chi-Square test. t1.29

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t2.1 **Table 2**
t2.2 Data of multifocal electroretinogram (mfERG) in cannabis users versus controls.

t2.3			Cannabis (n = 49)	Controls (n = 21)	p-Value
t2.4	<2° *(ring 1)*	N2 Implicit Time (ms)[a,b]	71,90 [70,30:73,35]	70,50 [69,20:72,20]	0,037
t2.5	2–5° *(ring 2)*	N1 Amplitude (μV)[a,b]	−232,50 [−264,00:−183,00]	−248,00 [−318,50:−210,50]	0,044
t2.6		P1 Implicit Time (ms)[a,b]	46,15 [45,00:46,95]	45,60 [43,90:46,25]	0,046
t2.7		N2 Implicit Time (ms)[a,b]	66,55 [64,30:70,80]	64,85 [62,90:65,70]	0,018
t2.8	5–10° *(ring 3)*	N1 Implicit Time (ms)[a,b]	24,90 [24,40:26,05]	24,40 [23,70:24,95]	0,034
t2.9		P1 Implicit Time (ms)[a,b]	43,75 [43,00:44,55]	42,65 [41,90:43,45]	0,006
t2.10	10–15° *(ring 4)*	P1 Implicit Time (ms)[a,b]	42,85 [42,20:43,70]	42,25 [41,65:42,65]	0,014

t2.11 Data are presented as median and interquartile range.
t2.12 [a] Quantitative variable represented as median and interquartile range.
t2.13 [b] Mann-Whitney *U* test.

66.65 ms [64.30: 70.80] in cannabis users versus 64.84 ms [62.90: 65.70] in controls. This difference was significant between groups (p = 0.018) (Fig. 3C). The median and IQR of N1 amplitude was −232.50 uV [−264.00: −183.00] in cannabis users versus −248.00 uV [−318.50: −210.50] in controls. This difference was significant between groups (p = 0.044).

In ring 3 (5–10°), the median and IQR of N1 implicit time was 24.90 ms [24.40: 26.05] in cannabis users versus 24.40 ms [23.70: 24.95] in controls. This difference was significant between groups (p = 0.034) (Fig. 3D). The median and IQR of P1 implicit time was 43.75 ms [43.00: 44.55] in cannabis users versus 42.65 ms [41.90: 43.45] in controls. This difference was significant between groups (p = 0.006) (Fig. 3E).

In ring 4 (10–15°), the median and IQR of P1 implicit time was 42.85 ms [42.20: 43.70] in cannabis users versus 42.25 ms [41.65: 42.65] in controls. This difference was significant between groups (p = 0.014) (Fig. 3F).

In ring 5 (>15°), no effect was significant.

3.3. Correlations

Among the 49 regular cannabis users, there was no significant correlation (Spearman Rank correlation) between the implicit time of N1, P1, N2 and alcohol use parameters (average alcohol consumption/week and AUDIT score). There were significant correlations (Spearman rank correlation) between N1 amplitude (2–5°) and the average of alcohol consumption/week (p = 0.02) and AUDIT score (p = 0.018). There

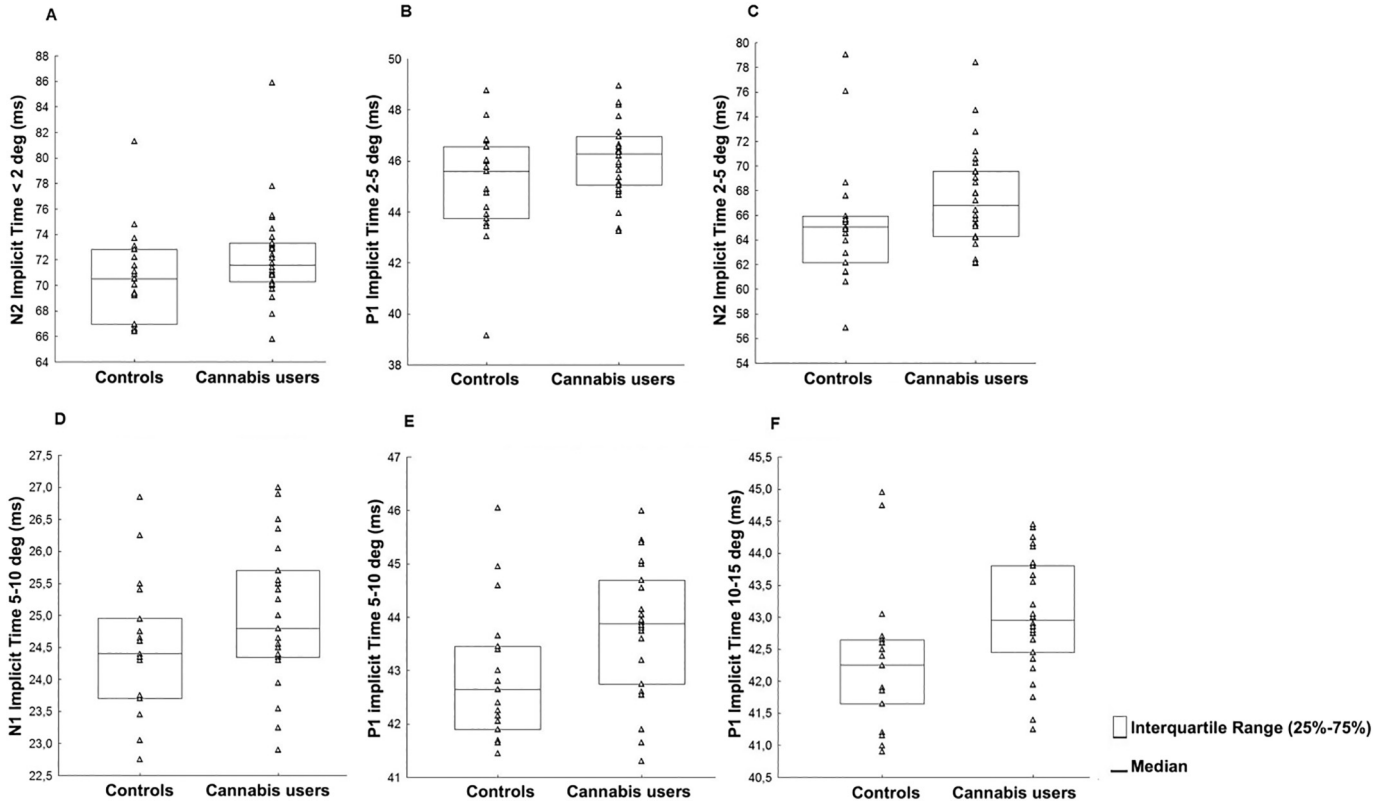

Fig. 3. A. Box plot of multifocal electroretinogram (mfERG) N2 implicit time (ms) in ring 1 (<2°), for cannabis users (n = 49) and controls (n = 21) with medians. Cannabis users showed increased implicit time and the difference between the groups is significant (p = 0.037). B. Box plot of multifocal electroretinogram (mfERG) P1 implicit time (ms) in ring 2 (2–5°), for cannabis users (n = 49) and controls (n = 21) with medians. Cannabis users showed increased implicit time and the difference between the groups is significant (p = 0.046). **C.** Box plot of multifocal electroretinogram (mfERG) N2 implicit time (ms) in ring 2 (2–5°), for cannabis users (n = 49) and controls (n = 21) with medians. Cannabis users showed increased implicit time and the difference between the groups is significant (p = 0.018). D. Box plot of multifocal electroretinogram (mfERG) N1 implicit time (ms) in ring 3 (5–10°), for cannabis users (n = 49) and controls (n = 21) with medians. Cannabis users showed increased implicit time and the difference between the groups is significant (p = 0.034). E. Box plot of multifocal electroretinogram (mfERG) P1 implicit time (ms) in ring 3 (5–10°), for cannabis users (n = 49) and controls (n = 21) with medians. Cannabis users showed increased implicit time and the difference between the groups is significant (p = 0.006). F. Box plot of multifocal electroretinogram (mfERG) P1 implicit time (ms) in ring 4 (10–15°), for cannabis users (n = 49) and controls (n = 21) with medians. Cannabis users showed increased implicit time and the difference between the groups is significant (p = 0.014).

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were significant correlations between the number of cigarettes per day and N1 (5–10°) implicit time ($p = 0.05$), P1 (10–15°) implicit time ($p = 0.036$), and N2 (<2°) implicit time ($p = 0.01$). No correlation was found between mfERG responses and cannabis use.

4. Discussion

The results show that for the regular cannabis users there was a significant increase in implicit times (+1 to 2 ms) for N2 (<2°), N2 and P1 (2–5°), P1 and N1 (5–10°) and P1 (10–15°). An isolated decrease in the amplitude of N1 (2–5°) was also found. We observed that implicit time was altered in all retinal areas from <2° to 10–15° with a major effect found in the central retina. This indicates that there is a delay in the transmission of the signal by the cones system, mainly by the ON and OFF bipolar cells, in retinal areas from the central retina – the macula and macular region – to the near periphery in regular cannabis users compared to the healthy group.

We have shown here, in relation to the previous results, that the central retina and its near periphery are preferentially impacted by the regular use of cannabis. This can be explained by the fact that the cones system is detected with a high cellular density in this area (Hoon et al., 2014), which explains why the majority of anomalies are observed there. This retinal area is crucial since it is used for sharp, precise and detailed vision. Thus, these anomalies could underlie potential alterations in precise, detailed and color vision in regular cannabis users, which may be supported by animal studies. In cones, endocannabinoids are largely detected in mammal and non-mammal species and play a crucial role in regulating light responses (Schwitzer et al., 2016; Yazulla, 2008). In goldfish, cones are endowed with a functional cannabinoid system including receptors, ligands, and enzymes (Yazulla, 2008). Using whole-cell patch-clamp recordings from cones in the goldfish retina, Struik et al., showed that cannabinoid agonists, through an action on cannabinoid receptors CB1, speed up the dynamic of the phototransduction deactivation cascade, resulting in an enhanced photosensitivity (Struik et al., 2006). In goldfish retinal cones, CB1 agonists -especially an endocannabinoid ligand named 2-arachidonoyl glycerol-, participate to modulate cone membrane currents by acting on CB1 receptors, in interaction with dopaminergic D2 agonists (Fan and Yazulla, 2003, 2004, 2005, 2007). These results were confirmed by electrophysiological measurements such as ERG. In mice lacking CB2 receptors, b-wave amplitudes recorded under photopic conditions necessitated more light adaptation time to reach stable values (Cecyre et al., 2013). Anomalies in this area have been detected in other neuropsychiatric disorders. In Parkinson's disease, for example, which is associated with a dopamine deficiency, a pathological elongation of P1 implicit times, mainly in the foveal and para-foveal areas, has been observed using mfERG in patients who did not yet have any visual symptoms (Moschos et al., 2012). Similar results were found here following regular cannabis use also leading to a dopamine deficiency. Interestingly, in Parkinson's disease, the decrease in N95 amplitude of the PERG has been correlated with a reduced quality of life as observed with a lower score of the Schwab and England Activities of Daily Living Scale (SE-ADL) (Garcia-Martin et al., 2014). These findings are of potential interest because a reduction in the quality of life has been observed among regular cannabis users (Aspis et al., 2015). It would be useful to see whether mfERG parameters can predict quality of life in cannabis users.

Our results back up and build on the results obtained using fERG and PERG that found a delay in the responses of bipolar (~1 ms) and ganglion cells (~6 ms) in regular cannabis smokers compared to healthy subjects (Schwitzer et al., 2017, 2018). However, considering the relative small delays observed with fERG and mfERG, the large delay observed with the PERG cannot originate exclusively from photoreceptor or bipolar cells but rather from the ganglions cells themselves. These results support the hypothesis that regular cannabis use may alter the functional properties of retinal cells in responding to various light stimulations by altering retinal neurotransmission (Schwitzer et al., 2015, 2016; Yazulla, 2008). This hypothesis is reinforced by the fact that cannabinoid receptors are present in the retina, especially in the cones system – the cones and their bipolar cells. This system is involved in the retina in regulating the synaptic release of neurotransmitters by different types of mechanisms. Cannabinoid agonists, for example, induce in animals a dose-dependent and reversible modulation of potassium, calcium and chloride currents in bipolar cells and cones (Schwitzer et al., 2015, 2016; Yazulla, 2008). As a result of these actions, the cannabinoid system can modulate the release of several neurotransmitters such as dopamine and glutamate (Middleton and Protti, 2011; Opere et al., 2006; Schlicker et al., 1996; Straiker and Sullivan, 2003; Weber and Schlicker, 2001), two neurotransmitters involved in the effects of cannabis on the central nervous system. Similarly, THC in the retina may inhibit the activity of the enzymes regulating neurotransmitter rates such as monoamine oxidase, thereby altering the level of the monoamines (Gawienowski et al., 1982). Accordingly, by modifying the synaptic release of dopamine and glutamate in the retina, the THC contained in cannabis joints might alter the depolarization and hyperpolarization of the ON and OFF bipolar cells, resulting in delays in their responses and in the transmission of the signal. Interestingly, these regulatory mechanisms – modulations of ionic currents and neurotransmission release – play a critical role in physiological processes in the retina such as phototransduction. As a consequence, we assume a role of exocannabinoids in the disruption of visual processing already when light penetrates in the eye, during phototransduction. Then, we suppose that these alterations at the first stages of visual processing occurring in the retina may have critical consequences in last and more integrated stages of visual processing.

There is a major difficulty in studying the effects of cannabis on the central nervous system: namely, the co-occurrence of other substances such as tobacco and alcohol (Meier et al., 2012). Since tobacco is frequently used in combination with cannabis in joints (Agrawal et al., 2012), it is difficult to distinguish the specific effects of each substance on retinal function except with a tobacco-only control group. The effect of regular tobacco use on retinal function has been seldom studied to date. A recent investigation evaluated the effects of active smoking on the retina using mfERG compared to a passive-smoking population (El-Shazly et al., 2017). The results showed that, among active smokers, there was a significant decrease in the P1 amplitude (<2°), an increase in P1 implicit times (<2°) and lower amplitude ratios. However, the comparison was not undertaken with a group of healthy subjects. Nevertheless, these results differ from those we observed among regular cannabis users (an increase in implicit times for N2 (<2°), N2 and P1 (2–5°), P1 and N1 (5–10°) and P1 (10–15°) and an isolated decrease in the amplitude of N1 (2–5°)). Alcohol is another psychoactive substance that is frequently consumed with cannabis among users (Degenhardt et al., 2008). Our two groups of participants – cannabis users and controls – showed a significant difference between the two populations for the AUDIT score but no significant difference in the number of units of alcohol consumed per week. As the number of units per week seems more clinically relevant for reflecting the toxicity of the alcohol and the AUDIT score for measuring at-risk use, we considered that alcohol use was non-significant in this analysis. Cannabis had an effect on implicit times, whereas alcohol appears to affect the amplitude of the N1. An increase in the magnitude of the retinal background noise was observed in users with co-occurrent consumption of cannabis and alcohol (Lucas et al., 2018). This supports the fact that alcohol use seems to be associated with modulation of wave's amplitude. It should be noted that we used DTL electrodes, with which the amplitudes can be reduced by 30 to 50% compared to those measured with scleral shells (Donald C Hood et al., 2012). This might also have contributed to the isolated decrease in the N1 amplitude (2–5°). Nevertheless, DTL electrodes are more tolerable and bearable than scleral shells for patients with psychiatric and addictive disorders since they are less invasive.

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DTL electrodes also help to prevent the eye anesthesia that could affect the quality of the signals collected.

Future studies should compare these results with those obtained among tobacco and/or alcohol users in order to clarify the effect each substance has on retinal function. Future research should also be combined with molecular studies to validate or invalidate the hypotheses of neurotransmission anomalies formulated here. Since the retina is an integral part of the SNC equipped with a complex neurotransmission system, these studies may improve our knowledge about synaptic modulations in the brain as a result of regular cannabis use.

Conflict of 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.

Contributors

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