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ORIGINAL RESEARCH

Use of a Novel EEG-Based Objective Test, the Cognalyzer®, in Quantifying the Strength and Determining the Action Time of Cannabis Psychoactive Effects and Factors that May Influence Them Within an Observational Study Framework

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ABSTRACT

Introduction: Current methods to detect recent delta-9-tetrahydrocannabinol (THC) use cannot objectively quantify its psychoactive effects (PE). The Cognalyzer®, an electroencephalography (EEG)-based method, detects and quantifies the strength of THC-induced PE on a scale from 0 to 100%. This study assesses the relationship between the magnitude of Cognalyzer® PE predictions and reported subjective drug effects for 4-h post-cannabis inhalation.

Methods: Seventy-five participants were enrolled in the study. Prior to ad libitum cannabis inhalation, an EEG recording episode was completed. Immediately after inhalation, the Drug Effects Questionnaire (DEQ) was administered and another EEG recording performed. For 25 participants, the study ended. For 50 participants, assessments were repeated at 30-min intervals for 4 h. EEG files were blinded and analyzed using two versions of the Cognalyzer® algorithm. The relationship between the Cognalyzer® PE level results and the DEQ was assessed using generalized linear models and multiple regression.

Results: There were significant PE increases from pre-cannabis for up to 3.5 h. Mean reports of feeling drug effects were > 0 at all post-inhalation time points ($p \leq 0.024$). Furthermore, there were significant relationships between the Cognalyzer® PE and self-reported perception of drug effects ($p \leq 0.001$). Subgroup analysis showed that Cognalyzer® PE levels were impacted by cannabis use history, subjective ratings of drug effects, oral fluid THC concentration and the cannabis product inhaled.

Conclusion: The findings show that the Cognalyzer® can be used to objectively determine the strength of cannabis psychoactive effects that cannabis products create on consumers and how it changes depending on their experience with cannabis. The Cognalyzer® can be used to conduct scientific consumer research to generate trustworthy informational material about the psychoactive experience of cannabis products. For clinical research, the Cognalyzer® can be used to study the pharmacodynamics of cannabinoids or delivery systems, such as nanoemulsifications.

Keywords: Cognalyzer®; THC effect; Psychoactive; Cannabis effects; EEG; Drug test

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Key Summary Points

Why carry out this study?

Current methods to detect recent delta-9-tetrahydrocannabinol (THC) use are limited in their ability to objectively quantify its psychoactive effects.

A novel method based on electroencephalography (EEG) developed by Zentrela Inc. for quantifying cannabis' psychoactive effects called the Cognalyzer® Test was investigated in this study.

This study assessed the relationship between the magnitude of Cognalyzer® psychoactive effects predictions and reported subjective drug effects for 4 h post-cannabis inhalation.

What was learned from the study?

The Cognalyzer® algorithm was able to generate objective psychoactive effect level predictions that were similar to the subjective Drug Effect Questionnaire ratings of level of 'HIGH'.

Having a tool that is able to objectively quantify psychoactive effects would enable researchers to investigate drug effects without reliance on subjective ratings.

The study findings can be applied to detecting and describing the time course of THC effects and used for informational, educational or product promotional purposes.

INTRODUCTION

Cannabis has therapeutic potential for a variety of indications, such as relief of chronic pain, treatment of anorexia, or relief of some symptoms of multiple sclerosis [1]. The recent legalization of recreational cannabis use in Canada as well as Georgia, Uruguay, South Africa, and 15 American states has preceded an international shift in policy and legislation towards decriminalization and legalization. As a result, legal recreational cannabis users are increasingly in need of reliable information on how their cannabis intoxication level can be estimated and managed. Although in Canada public health guidelines for decreasing risk from cannabis usage have been developed and disseminated [2], data suggest that 20–40% of Canadians are not complying with one or more of the guidelines [3]. More research is needed to understand the acute effects of cannabis intake and delta-9-tetrahydrocannabinol's (THC) psychoactive effects in ecologically valid paradigms. Typical cannabis research involves strictly controlled administration of precise doses of THC under conditions very different from how cannabis is usually consumed. In our study we have chosen an observational study framework where participants self-administer their chosen legally available retail product ad libitum. Using this more ecologically valid framework may be important to provide consumers with useful information to guide them in safe selection and use of legally available cannabis products, inform expected effects of strains or intake methods, and allow cannabis producers to differentiate their products using scientifically verifiable claims. With the advent of 'Cannabis 2.0' products in Canada (products other than traditional dried flower), various routes of administration such as edibles, capsules, extracts, and topicals are now available to consumers but their effects have not been widely studied. New delivery methods for beverages using nanoparticle delivery technology, which purports to speed onset of effects, have not been properly evaluated, yet these products are already available. Furthermore, much existing research in the USA has been conducted using only dried cannabis flower available through the National Institute on Drug Abuse (NIDA) drug programme [4], which contains a *maximum* content of 12% THC whereas 90% of cannabis seized by authorities up until 2015 has contained levels exceeding 20% THC [5]. In Colorado, legally available cannabis has an

average THC level of 18.7%, with some dried flower products containing 30% THC or more [6].

The pharmacokinetics of THC and its metabolites have been characterized [7–9]. Blood levels of THC peak within minutes when inhaled and generally decline by 90% within 2–3 h, depending on dosage. These kinetics differ between smoking and vaping methods of inhalation, with vaping producing higher concentrations of detected THC and metabolites in whole blood [8]. Oral preparations have a much slower time course, with peak concentrations of THC occurring at 3 h post consumption, and do not return to baseline for $\sim$ 22 h with a 50-mg dose [7]. Metabolites in blood take much longer to clear, being present in quantities $>$ 50% of peak after $\geq$ 400 min [10], and THC-COOH can be present in urine 93.3 h after ingestion of an oral dose [11]. These physiological levels, reported in the above studies on infrequent users who had abstained from recent cannabis use before testing, are highly dependent on frequency of cannabis use. In frequent cannabis users who reported daily or near daily use over the preceding 14 days, 6 of 25 participants had detectable THC after 6 days of monitored abstinence [12]. For frequent users, both THC and metabolite (11-OH-THC, THCCOOH) concentrations peak higher and last longer than for infrequent users [9]. These metabolic changes take some time to develop and are not yet shown after 10–12 days of daily dosing [13]. Furthermore, both subjective and performance effects of inhaled cannabis smoke do not appear to be necessarily dose dependent, although this may be partly explained by dose titration via changes in inhalation patterns [14]. In summary, the degree of subjective 'high' does not seem to be reliably related to plasma concentrations [15], but does depend on such factors as cannabis use history and route of administration.

Despite well-documented psychoactive effects (PE) of THC, early studies investigating the effect on brain function using electroencephalography (EEG), which records electrical brain activity using electrodes affixed to the scalp, have had mixed results. When studying 'resting EEG', where participants did not attend to or respond to specific stimuli or engage in tasks, cannabis intake either had no effect, increased alpha (8–13 Hz) power, decreased alpha power, decreased alpha frequency (Hz) or increased beta (13–30 Hz) power in resting EEG [16–19]. Increased alpha power is generally thought to correlate with relaxation whereas beta band activity is thought to index cortical excitation. Error-related negativities (ERNs), which are evoked EEG responses that occur in response to errors in speeded reaction tasks, were reduced in amplitude in the THC vs. placebo condition for regular THC users (at least two uses per week for the last year), although the behavioural performance was not significantly affected [20]. High-potency cannabis reduced magnitude of visually evoked event-related potentials in a visual selective attention task [21]. Lukas et al. [22] had participants continuously record mood state during intoxication using a joystick and found that short episodes of euphoria were accompanied by 70% increases in alpha (7–13 Hz) band power in electrodes P3 and P4 when the data were subdivided into 12-s epochs. These researchers theorized that the THC effect is not continuous in nature, but occurs in discrete bursts, a position that we propose to provide support for in this study. In a study where auditory stimuli amplitude modulated at 40 Hz were used to evoke an auditory steady-state (ASSR) EEG response in the gamma (40-Hz) band study, Cortes-Briones and colleagues showed that THC disrupted gamma band neural oscillatory activity in humans but interestingly not for 20- or 30-Hz induced oscillations [23]. This indicates that gamma band activity, which plays a key role in sensory integration, is somewhat disrupted by THC. Higher gamma band (50–80 Hz) oscillations may play a role in emotional state processing [24] and THC-induced disruptions in these bands may indicate a mechanism for mood changes resulting from cannabis intake. To summarize, there has been no consensus on the specific properties of the EEG affected by cannabis intake.

A novel EEG-based method developed by Zentrela Inc. for quantifying cannabis PE called the Cognalyzer® test was investigated in this study. Instead of measuring levels of THC in

oral fluid, urine, blood or breath, the Cognalyzer® quantifies cannabis PE by detecting the presence and consistency of THC effects using EEG measurement. It is a portable and objective solution that can be applied to workplace or roadside testing. The accuracy, sensitivity and specificity of two versions of the Cognalyzer® algorithms in distinguishing between EEG activity from pre- and post-inhalation of cannabis have been previously published [25]. The accuracy was 85.5% and 83.9%, sensitivity was 87.1% and 88.7% and specificity was 83.9% and 79.0% for the two algorithm versions, V1 and V2, respectively. For more information on the psychometric properties of our and other tests when used to determine potential cannabis impairment, please refer to our previous publication [25]. The objective of this study was to measure the time action curve of cannabis' psychoactive effects on a 0–100 scale for up to 4 h post-inhalation of cannabis. This time action curve of cannabis' PE can quantify the onset action time, maximum potency of PE and duration of action. Furthermore, we aimed to demonstrate that this action curve of cannabis' PE may be influenced by factors such as cannabis sensitivity of the participant and factors related to cannabis inhalation such as dosage or intake method.

METHODS

This study was conducted 28 February 2020 to 29 August 2020 in London, ON, Canada, at KGK Science Inc. clinic site. The study was approved by Institutional Review Board (IRB) Services, Aurora, Ontario, on 14 February 2020 and all participants provided written informed consent. The study was conducted in compliance with the Declaration of Helsinki guidelines and its subsequent amendments and International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) Guideline for Good Clinical Practice (GCP).

A complete description of the enrolment criteria, study methods and data blinding protocols have been published elsewhere [23]. The protocol was structured as an observational study; participants provided their own cannabis products and consumed ad libitum.

Participants were required to have a self-reported cannabis use history that included: use cannabis at least a few times/month and no more than 2–3 times/week confirmed by participants' self-report using a seven-item Cannabis Use Questionnaire; usually use cannabis via an inhalation route of administration (vape, smoke); do not struggle to control their high or get dizzy, vomit or become paranoid; can handle at self-reported 7/10 level of high (defined as "I feel THC's psychoactive effects in a steady and constant way BUT I can still walk and chat" on the Cannabis Use Questionnaire) and have not recently used recreational drugs other than alcohol or cannabis. These criteria were chosen on the basis of previous pilot studies. All participants brought a legally purchased inhalation cannabis product to the study clinic.

Study Procedures and Assessments

Screening of potential participants was conducted via telephone. At the in-clinic visit, participants signed an informed consent form, medical history and eligibility criteria were reviewed, and 75 participants between the ages of 19 and 65 years were enrolled. Study assessments were conducted before and after ad libitum cannabis inhalation following 3 days of abstinence from cannabis consumption. Prior to cannabis inhalation, seated blood pressure (BP) and heart rate (HR) were measured, oral fluid was collected (Quantisal™, Immunalysis), the EEG headset was applied, and photographs were captured to document electrode placements. An EEG data collection 'episode', which consisted of two 2.5-min baseline EEG recordings conducted one immediately after the other, was completed with the Cognalyzer® investigational device, conducted in an eyes-closed resting state. Participants were escorted outside the clinic for cannabis inhalation via smoking or vaping until a self-reported 7/10 feeling of high was achieved. Immediately upon finishing, a 5-item Drug Effects Questionnaire (DEQ-5) was administered and another EEG session of two 2.5-min post-cannabis inhalation

Cognalyzer® EEG readings was collected. The DEQ-5 (hereafter referred to as DEQ) is comprised of five items: FEEL, HIGH, DISLIKE, LIKE, and MORE. Each item was assessed with an 11-point 0–10 numeric rating scale. The scale ranged from 0 indicating 'not at all' to 10 indicating 'extremely' [26, 27]. Fifty participants remained in the clinic for 4 h post-cannabis and the Cognalyzer® and DEQ were repeated at approximately 30 min, 1, 1.5, 2, 2.5, 3, 3.5 and 4 h post-inhalation. A second oral fluid sample was collected after 4 h.

Participants' vital signs were measured, and they were assessed for safety to leave the clinic at the end of the study visit. All adverse events (AE) were documented in the study record and classified as per the description, duration, intensity, frequency and outcome. Causality and intensity were determined by the medical directory as appropriate. The Medical Dictionary for Regulatory Activities terminology (MEDRA) System Organ Class, version 22.0, was used to code AEs.

Investigational Device

The Cognalyzer® is a new method to detect the presence of cannabis' PE based on brain signal analysis and machine learning that has been developed by Zentrela Inc. The EEG device is an 8-channel system with a 250-Hz sampling rate and 24-bit resolution with a proprietary portable data collection device. Data files can be analysed in real time or saved for later analysis as done in this study. Ten electrodes were placed on left and right side of frontal, temporal, occipital and parietal lobes and on the forehead (ground and DC Bias Drive) and held in position by a proprietary electrode headband. This headband positioned six of the electrodes along a strap situated approximately around the Fz to Oz line, with two additional electrodes positioned above the strap at the rear of the head at approximately P3/P4 positions. Conductive gel was applied to reduce electrode impedance < 35 kΩ. During the EEG collection, data were segmented into 10-s segments with 5-s overlap, and each segment was immediately analyzed for artefacts such as peak voltage exceeding a threshold of $\pm$ 500 μV or excessive amounts of energy at 60 Hz. Data collection continued until 30 artefact-free segments were collected.

Data and Statistical Analysis

Algorithm Description and Calibration

The Cognalyzer® algorithm is patent-pending and is applied to the collected EEG data. Each segment is independently analyzed for features including power spectral density, cross power spectral density, coherence, and root-mean-square (RMS) power. The algorithm produces a classification for each segment of data, either 'normal' or 'abnormal', and the strength of the PE is determined by calculating the percentage of 'abnormal' segments (0–100%). The measured PE level of an episode, which consists of two $\sim$ 2.5-min long continuous data recordings, is the average of the percentage of total segments classified as abnormal from the artefact-free segments identified in the two EEG files recorded in that episode. The results from two candidate algorithms using slightly different parameter weightings were evaluated, Cognalyzer® version 1 (V1) and version 2 (V2). All EEG data files were blinded by KGK Science Inc. prior to sending to Zentrela Inc. for analysis [28].

To account for the varying individual PE sensitivities, the algorithm V1 was calibrated for each participant based on the result of the pre-cannabis episode. This process was applied to reduce false positives and negatives for each participant. The calibration process reduced the sensitivity of the algorithm if the 'pre-cannabis inhalation' episode had a very high PE result and increased it if it had a very low result. The threshold parameter used to adjust the sensitivity and specificity of the classifier is defined in the algorithm. This calibration process required the unblinded information of the pre-cannabis episode files only for each session and was conducted after the blinded analysis was completed. No information from the post-cannabis inhalation episodes was used for calibration.

Upon review of data, there was variability of the exact timing of data collection relative to

the time the participant completed ad libitum cannabis inhalation, which was designed to occur at nominal 30-min intervals. To eliminate the variability from this ideal, each participant's actual session timing and effect level was fitted with a cubic spline function and interpolated at 5-min intervals before averaging and statistical analyses of group data. This method was only employed in the subgroup analyses.

Statistical Analysis

Cognalyzer® and oral fluid THC levels were summarized as arithmetic means with standard deviations. Within-group analyses were conducted using the paired Student's *t*-test or Wilcoxon signed rank test, as appropriate. For the 50 participants who completed the 4-h study visit, the relationship between log transformed Cognalyzer® THC measurements and DEQ responses at 0 h, 0.5 h, 1 h, 1.5 h, 2 h, 2.5 h, 3 h, 3.5 h and 4 h were assessed using repeated measures mixed models. DEQ questions were the dependent variables, with Cognalyzer® THC measurements by time as fixed effects and subject as random effect.

The calibration method was evaluated using a linear correlation between DEQ question 1 ("Do you feel a drug effect, right now?") and the Cognalyzer® predicted PE before and after calibration.

Seven subgroup analyses were conducted post hoc, using the calibrated and time adjusted data for algorithm V2. The subgroups were selected based on properties that may have had an effect on the PE curve. The following subgroups were analyzed: (1) pre-inhalation oral fluid test $\geq$ 5 ng/ml vs. $<$ 5 ng/ml; (2) final oral fluid test at 30 ng/ml (median split); (3) DEQ Q1 score at 30 min, corresponding to the typical peak of psychoactive effects observed after inhalation as participants had been instructed to inhale cannabis until this level had been achieved (split at DEQ = 6); (4) DEQ Q1 score at 4 h (median split); (5) inhalation method (6) weekly reported cannabis use (3 groups); (7) indica or sativa product.

All hypothesis testing was carried out at the 5% (2-sided) significance level unless otherwise specified. p values were rounded to three decimal places. p values < 0.001 were reported as < 0.001 and those ≤ 0.05 were considered statistically significant. All analyses were performed using R Statistical Package version 3.6.3 (R Core Team, 2020) for Microsoft Windows.

RESULTS

Participants

Participants in this study were aged 29.84 ± 8.01 (19–55) years, 64% male and 36% female. Cannabis use histories were variable between participants and ranged from 3 months to 29 years. The youngest age of onset of use was 12 years old. Participants reported using cannabis 2.38 ± 0.75 times/week. Reported frequencies of use were 2–3 times/week for 74.7% of participants and a few times per month for 25.3%. There were 104 participants screened, 75 enrolled and 72 completed the full visit; three participants dropped out of the study before completion. Of the 75 enrolled participants, 25 were planned to complete only the first two time points (before and immediately after consumption) while the remaining 50 were to complete multiple EEG measures at 30-min intervals for the 4 h post inhalation time frame.

The per-protocol (PP) population, $n = 62$, consisted of participants who had completed the full study visit and all procedures connected with the Cognalyzer® EEG measurement and had two EEG files for the time points before and immediately after cannabis consumption. These participants were used to complete the psychometric evaluation of the ability of the algorithm to detect recent cannabis use. Forty-one participants had two EEG measurements at every 30-min interval for the 4-h post-cannabis time frame.

Relationship Between Cognalyzer® and DEQ

Pre-cannabis predicted PE levels were 18 ± 24% and 18 ± 30% for Cognalyzer® V1 and V2

algorithms, respectively. As shown in Fig. 1, immediately post-inhalation, the predicted PE levels significantly increased to 73 ± 30% and 78 ± 30% for Cognalyzer® V1 and V2, respectively ($p < 0.001$). Cognalyzer PE levels receded gradually to ~ 40% over the 4-h measurement period. With both versions of the Cognalyzer®, increases in PE level predictions from pre-cannabis were significant at all post-cannabis time points ($p \leq 0.003$) (Table 1).

Immediately post-cannabis participants reported an average 7.58/10 (SD ± 0.97) on feeling the effects of the drug (Q1), 7.48/10 (SD ± 0.72) on feeling high (Q2) and 6.84/10 (SD ± 2.03) on liking the effects (Q4) (Table 2). There were significant reductions in feeling the drug effects and being high from 30-min post-cannabis inhalation ($p \leq 0.011$). After 1.5 h there was significant reduction in liking the effects ($p \leq 0.011$). Participants reported scores of 1.74/10 (SD ± 2.10) on disliking the effects of the drug (Q3) immediately following cannabis inhalation. There were significant reductions in the scores 1.5-, 3- and 4-h post-consumption ($p \leq 0.032$). Participants reported scores of 2.23/10 (SD ± 2.65) on wanting more of the drug (Q5) that did not change over the 4-h post-cannabis.

The relationships between Cognalyzer® predicted log PE level, DEQ and time difference between V1 and V2 of the Cognalyzer® algorithm (Tables 3, 4). With V1 of the Cognalyzer® algorithm, there was a significant relationship between the Cognalyzer® predicted log PE level and subjective reports of liking the drug effects (DEQ Q3) ($p = 0.002$); this relationship was not significant with Cognalyzer® V2. The relationship between log PE prediction and subjective report of wanting more of the drug (DEQ Q5) was significant with V2 of the Cognalyzer® algorithm ($p = 0.001$).

There were significant relationships observed between time and feeling the effects of the drug (Q1) from 0.5 to 4 h post-inhalation ($p \leq 0.045$) and liking the effects from 1.5 to 4 h ($p \leq 0.048$), indicating that the DEQ scores were significantly affected by the cannabis inhalation. Significance between time and feeling high (Q2) and disliking the drug effects (Q3) differed between V1 and V2. There were no significant relationships between time and wanting more of the drug (Q5) for either algorithm.

Interactions between time and predicted log PE level from 3.5 to 4 h ($p \leq 0.028$) for feeling the drug effects (Q1) and feeling high (Q2) were significant with V1 only. There were significant interactions between log PE prediction and time for disliking the drug effects (Q3) for both algorithm versions ($p \leq 0.049$). Interactions were significant for liking the drug effects from 2 to 4 h with V1 ($p \leq 0.025$) and for wanting more of the drug from 0.5 to 3 h and 4 h with V2 ($p \leq 0.038$).

Participant Calibration

With the calibration method there was an increase in the goodness of fit of a linear correlation between DEQ Q1 and the Cognalyzer® PE from algorithm V1 (see Fig. 2). The coefficient of determination was $r^2 = 0.064$ before calibration and $r^2 = 0.204$ after, implying that the calibration procedure produced a better fitting model of the data.

Subgroup Analysis

Pre- and Post-Inhalation Oral Fluid THC Test

In a post hoc analysis, pre-cannabis oral fluid THC concentrations of < 5 ng/ml ($n = 32$) and ≥ 5 ng/ml ($n = 9$) were used to divide the participants into subgroups (Fig. 3a). There were no significant between-group differences in the subgroups. Participants with a negative (< 5 ng/ml) pre-cannabis oral fluid THC test had significant within-group increases in PE at all post-cannabis time points ($p \leq 0.001$). Participants with higher pre-cannabis THC concentration had significant increases in PE up to 3.5 h.

A second subgroup analysis was based on oral fluid THC concentrations 4 h post-cannabis inhalation of < 30 ng/ml ($n = 21$) and ≥ 30 ng/ml ($n = 20$) (Fig. 3b). Between groups, at $t = 60$ min, the high oral fluid THC concentration group had significantly greater PE than the low group ($p \leq 0.045$). Conversely to the pre-inhalation oral fluid subgroups, in both post-inhalation groups there were significant within-

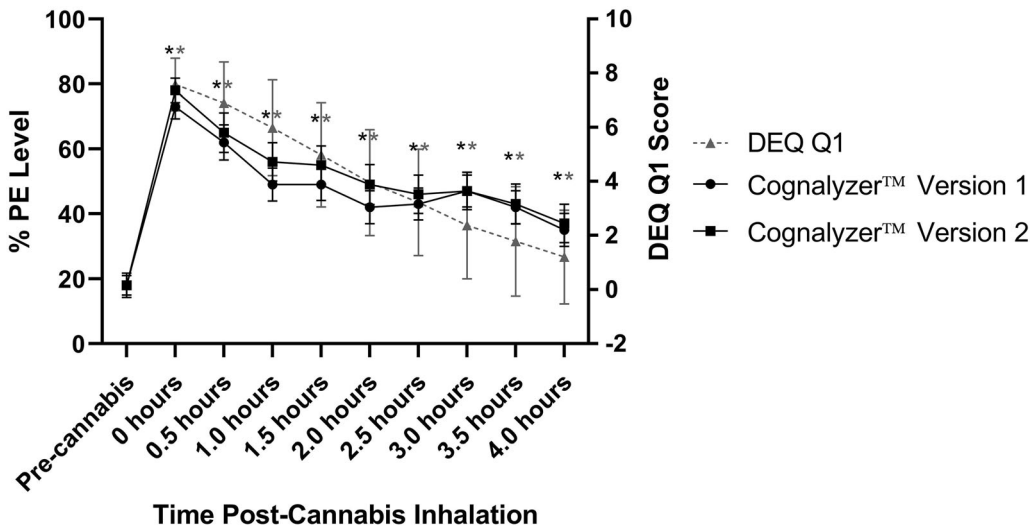


Fig. 1 PE predictions for versions 1 and 2 of the Cognalyzer® and DEQ scores for Q1: do you feel the drug effects right now over time? Values presented as mean ± standard error of the mean (SEM); $^{*}p < 0.05$. DEQ Q1 (right scale) indicates the results from question 1 of the Drug Effects Questionnaire. The black asterisks indicate that the Cognalyzer V1 score is significantly elevated from the baseline; the grey asterisks indicate the same for the Cognalyzer V2 score

group increases in PE at all time points ($p \leq 0.004$). A post hoc *t*-test on the difference in DEQ FEEL ratings between the two groups found no significant difference ($t = -1.006$, $p = 0.323$, n.s).

DEQ Q1 Score at 30 Min and 4 H

Thirty minutes post-inhalation, participants were grouped based on the DEQ score on 'Q1: How high do you Feel' of ≤ 6 ($n = 12$) and > 6 ($n = 29$) (Fig. 3c). Between groups, the increase PE at 30–120 min was greater in participants with higher 30-min DEQ ($p \leq 0.031$). Within groups, participants with a higher DEQ rating had significantly greater PE at all post-cannabis time points ($p \leq 0.001$). Participants with a lower DEQ feeling of high had significant increases only until 75 min post-cannabis ($p \leq 0.038$).

Four hours post-inhalation, the subgroup analysis was based on DEQ Q1 score = 0 ($n = 21$) and ≥ 1 ($n = 20$) (Fig. 3d). Between groups, the increase in PE at 75 min was greater in participants with higher DEQ ($p = 0.021$). Within both groups participants had significantly greater PE at all time points ($p \leq 0.003$).

Cannabis Inhalation Method

Another subgroup analysis was based on the method used for cannabis inhalation at the study visit. This analysis was conducted post hoc. The number of participants in each group was 3, 34 and 4 in the bong, smoking and vaping groups, respectively (Fig. 3e). There were within-group increases in %PE prediction at 30 min in the bong group ($p = 0.025$) and at all time points in the smoking group ($p \leq 0.001$). There were no significant within-group changes in the vaping group.

Indica vs. Sativa Product

The post hoc subgroup analysis was based on indica ($n = 11$) or sativa ($n = 20$) inhaled at the study visit (Fig. 3f). There were no significant between-group differences and there were significant within-group increases in PE for all times points with both indica and sativa products ($p \leq 0.013$).

Weekly Reported Frequency of Cannabis Use

Post hoc, participants were grouped based on self-reported frequency of cannabis use of ≤ 1 time/week ($n = 10$) and ≥ 2 times/week ($n = 30$)

Table 1 Cognalyzer® psychoactive effect (PE) level predictions at pre-cannabis and 0 h, 0.5 h, 1 h, 1.5 h, 2 h, 2.5 h, 3 h, 3.5 h, 4 h post-cannabis; change in Cognalyzer® PE level predictions from pre-cannabis to 0 h, 0.5 h, 1 h, 1.5 h, 2 h, 2.5 h, 3 h, 3.5 h, 4 h post-cannabis in the PP population (n = 41). For the pre-cannabis and 0 h post-cannabis tests, n = 62, indicated by an asterisk

Cognalyzer® PE Level Predictions						
Study Timepoint	**Cognalyzer® Version 1 PE Level Mean ± SD (n) Median (Min - Max)**	**Cognalyzer® Version 1 PE Level Change from Pre-cannabis**	**Within-Group P-Value**	**Cognalyzer® Version 2 PE Level Mean ± SD (n) Median (Min - Max) Within-Group P-Value**	**Cognalyzer® Version 2 PE Level Change from Pre-cannabis**	**Within-Group P-Value**
Pre-cannabis	0.18 ± 0.24 (62)* 0.06 (0.00 to 0.98)	-	-	0.18 ± 0.30 (62)* 0.02 (0.00 to 1.00)	-	-
0 hr Post-cannabis	0.73 ± 0.30 (62)* 0.88 (0.00 to 1.00)	0.55 ± 0.33 (62)* 0.65 (-0.45 to 1.00)	<0.001 (w)	0.78 ± 0.30 (62)* 0.92 (0.00 to 1.00)	0.61 ± 0.36 (62)* 0.72 (-0.08 to 1.00)	<0.001 (w)
0.5 hr Post-cannabis	0.62 ± 0.35 (41) 0.72 (0.00 to 1.00)	0.41 ± 0.36 (41) 0.45 (-0.50 to 1.00)	<0.001 (w)	0.65 ± 0.39 (41) 0.83 (0.00 to 1.00)	0.43 ± 0.44 (41) 0.50 (-0.67 to 1.00)	<0.001 (w)
1 hr Post-cannabis	0.49 ± 0.33 (41) 0.50 (0.00 to 1.00)	0.28 ± 0.39 (41) 0.22 (-0.78 to 0.88)	<0.001 (w)	0.56 ± 0.38 (41) 0.65 (0.00 to 1.00)	0.33 ± 0.48 (41) 0.18 (-0.87 to 0.97)	<0.001 (w)
1.5 hr Post-cannabis	0.49 ± 0.32 (41) 0.55 (0.00 to 1.00)	0.28 ± 0.33 (41) 0.25 (-0.37 to 0.98)	<0.001 (w)	0.55 ± 0.38 (41) 0.65 (0.00 to 1.00)	0.32 ± 0.44 (41) 0.23 (-0.62 to 1.00)	<0.001 (w)
2 hr Post-cannabis	0.42 ± 0.33 (41) 0.38 (0.00 to 1.00)	0.21 ± 0.32 (41) 0.18 (-0.50 to 0.82)	<0.001 (w)	0.49 ± 0.40 (41) 0.60 (0.00 to 1.00)	0.27 ± 0.39 (41) 0.10 (-0.72 to 1.00)	<0.001 (w)
2.5 hr Post-cannabis	0.43 ± 0.32 (41) 0.35 (0.00 to 1.00)	0.22 ± 0.28 (41) 0.15 (-0.42 to 0.95)	<0.001 (w)	0.46 ± 0.38 (41) 0.42 (0.00 to 1.00)	0.24 ± 0.38 (41) 0.10 (-0.59 to 0.95)	<0.001 (w)
3 hr Post-cannabis	0.47 ± 0.32 (41) 0.42 (0.00 to 1.00)	0.26 ± 0.30 (41) 0.25 (-0.55 to 0.98)	<0.001 (w)	0.47 ± 0.37 (41) 0.55 (0.00 to 1.00)	0.24 ± 0.39 (41) 0.13 (-0.82 to 0.98)	<0.001 (w)
3.5 hr Post-cannabis	0.42 ± 0.33 (41) 0.32 (0.00 to 0.98)	0.21 ± 0.30 (41) 0.15 (-0.35 to 0.92)	<0.001 (w)	0.43 ± 0.40 (41) 0.40 (0.00 to 1.00)	0.21 ± 0.40 (41) 0.10 (-0.50 to 1.00)	0.001 (w)
4 hr Post-cannabis	0.35 ± 0.33 (41) 0.20 (0.00 to 0.98)	0.14 ± 0.27 (41) 0.08 (-0.40 to 0.85)	0.003 (w)	0.37 ± 0.38 (41) 0.13 (0.00 to 1.00)	0.15 ± 0.32 (41) 0.03 (-0.55 to 0.90)	0.002 (w)

n, number of participants; min, minimum; max, maximum, SD, standard deviation
Within-group p-values generated using the paired Student's t test or Wilcoxon Signed Rank test, denoted by (w)
62 participants were included in the pre- and immediately post-cannabis analysis

Within-group p values generated using the paired Student's t-test or Wilcoxon signed rank test, denoted by (w); *62 participants were included in the pre- and immediately post-cannabis analysis
n number of participants, *min* minimum, *max* maximum, *SD* standard deviation

Table 2 Modified drug effects questionnaire (DEQ) scores at 0 h, 0.5 h, 1 h, 1.5 h, 2 h, 2.5 h, 3 h, 3.5 h, 4 h post-cannabis; change in DEQ scores from 0 h post-cannabis to 0.5 h, 1 h, 1.5 h, 2 h, 2.5 h, 3 h, 3.5 h, 4 h post-cannabis in the PP population (n = 41). For the pre-cannabis and 0 h post-cannabis tests, n = 62, indicated by an asterisk

Modified Drug Effects Questionnaire (DEQ)															
Study Timepoint	QUESTION 1 FEEL			QUESTION 2 HIGH			QUESTION 3 DISLIKE			QUESTION 4 LIKE			QUESTION 5 MORE		
	Mean ± SD (n)	Change	P-Value	Mean ± SD (n)	Change	P-Value	Mean ± SD (n)	Change	P-Value	Mean ± SD (n)	Change	P-Value	Mean ± SD (n)	Change	P-Value
0 hr Post-cannabis	7.58 ± 0.97 (62)*	-	-	7.48 ± 0.72 (62)*	-	-	1.74 ± 2.10 (62)*	-	-	6.84 ± 2.03 (62)*	-	-	2.23 ± 2.65 (62)*	-	-
0.5 hr Post-cannabis	6.90 ± 1.51 (41)	-0.59 ± 1.34 (41)	0.011 (w)	6.78 ± 1.42 (41)	-0.71 ± 1.10 (41)	<0.001 (w)	1.66 ± 2.27 (41)	-0.15 ± 1.67 (41)	0.268 (w)	6.98 ± 2.16 (41)	0.15 ± 1.33 (41)	0.746 (w)	2.32 ± 2.81 (41)	0.07 ± 1.57 (41)	1.000 (w)
1 hr Post-cannabis	5.98 ± 1.77 (41)	-1.51 ± 1.45 (41)	<0.001 (w)	5.85 ± 1.74 (41)	-1.63 ± 1.46 (41)	<0.001 (w)	1.44 ± 2.17 (41)	-0.37 ± 1.32 (41)	0.089 (w)	6.46 ± 2.41 (41)	-0.37 ± 1.61 (41)	0.194 (w)	2.12 ± 2.62 (41)	-0.12 ± 1.85 (41)	0.753 (w)
1.5 hr Post-cannabis	4.98 ± 1.93 (41)	-2.51 ± 1.66 (41)	<0.001 (w)	4.68 ± 1.89 (41)	-2.80 ± 1.60 (41)	<0.001 (w)	1.27 ± 2.16 (41)	-0.54 ± 1.69 (41)	0.032 (w)	5.93 ± 2.45 (41)	-0.90 ± 2.30 (41)	0.011 (w)	2.02 ± 2.47 (41)	-0.22 ± 2.36 (41)	0.953 (w)
2 hr Post-cannabis	3.95 ± 1.96 (41)	-3.54 ± 1.90 (41)	<0.001 (w)	3.68 ± 2.02 (41)	-3.80 ± 1.82 (41)	<0.001 (w)	1.41 ± 2.22 (41)	-0.39 ± 2.00 (41)	0.183 (w)	5.61 ± 2.68 (41)	-1.22 ± 2.72 (41)	0.002 (w)	1.93 ± 2.56 (41)	-0.32 ± 2.47 (41)	0.657 (w)
2.5 hr Post-cannabis	3.22 ± 1.97 (41)	-4.27 ± 1.96 (41)	<0.001 (w)	2.93 ± 2.11 (41)	-4.56 ± 1.95 (41)	<0.001 (w)	1.41 ± 2.33 (41)	-0.39 ± 2.15 (41)	0.209 (w)	4.78 ± 2.49 (41)	-2.05 ± 2.68 (41)	<0.001 (w)	2.07 ± 2.75 (41)	-0.17 ± 2.82 (41)	0.951 (w)
3 hr Post-cannabis	2.37 ± 1.97 (41)	-5.12 ± 2.06 (41)	<0.001 (w)	2.17 ± 2.10 (41)	-5.32 ± 1.99 (41)	<0.001 (w)	1.02 ± 2.09 (41)	-0.78 ± 2.16 (41)	0.013 (w)	4.02 ± 3.00 (41)	-2.80 ± 3.49 (41)	<0.001 (w)	2.17 ± 3.11 (41)	-0.07 ± 3.20 (41)	0.990 (w)
3.5 hr Post-cannabis	1.78 ± 2.02 (41)	-5.71 ± 2.09 (41)	<0.001 (w)	1.49 ± 2.04 (41)	-6.00 ± 1.94 (41)	<0.001 (w)	1.20 ± 2.50 (41)	-0.61 ± 2.93 (41)	0.056 (w)	3.56 ± 3.12 (41)	-3.27 ± 3.65 (41)	<0.001 (w)	2.17 ± 2.97 (41)	-0.07 ± 3.16 (41)	0.931 (w)
4 hr Post-cannabis	1.20 ± 1.74 (41)	-6.29 ± 1.91 (41)	<0.001 (w)	1.02 ± 1.74 (41)	-6.46 ± 1.72 (41)	<0.001 (w)	0.76 ± 1.61 (41)	-1.05 ± 2.37 (41)	0.003 (w)	3.20 ± 3.27 (41)	-3.63 ± 3.92 (41)	<0.001 (w)	2.07 ± 3.04 (41)	-0.17 ± 2.96 (41)	0.677 (w)

n, number of participants; min, minimum; max, maximum, SD, standard deviation

Within-group p-values generated using the paired Student's t test or Wilcoxon Signed Rank test, denoted by (w)

62 participants were included in the pre- and immediately post-cannabis analysis

Within-group p values generated using the paired Student's t-test or Wilcoxon signed rank test, denoted by (w); *62 participants were included in the pre- and immediately post-cannabis analysis

n number of participants, *min* minimum, *max* maximum, *SD* standard deviation

(Fig. 3g). There were no significant between-group differences and significant within-group increases in PE at all time points for all groups ($p \leq 0.021$).

DISCUSSION

The Cognalyzer® algorithm, employed in the manner described here, is able to both detect and quantify the PE of cannabis on brain activity measured using EEG after inhalation. When a threshold is applied to the calculated PE level for a participant, the algorithm is up to 85.5% accurate at blindly classifying whether a participant is currently experiencing an altered brain state [28]. This capability is comparable to the accuracy of current drug testing methods such as saliva or blood tests; however, these methods only detect the presence of the psychoactive substance indicating recent use and cannot directly quantify the magnitude of the PE. When the Cognalyzer algorithm is used to quantify PE, the mean level remains significantly elevated above pre-cannabis inhalation levels for up to 3.5 h. Furthermore, the PE level peaks at ~ 30–40 min after cannabis inhalation, and then gradually decreases over the subsequent 4 h, similar to reported subjective psychoactive effects (see, e.g., [7]). This peak, however, occurs notably more slowly than peak blood concentrations of THC after inhalation, which occur almost instantly [9].

Our derived measure is based on the percentage of short segments of EEG data discretely classified as 'abnormal' within a ~ 5-min data collection episode, objectively measuring the THC PE. Using this method, the onset time, potency (magnitude of PE at the peak) and duration of psychoactive effects induced by a dosage of inhaled cannabis can be calculated. This is different from the blood THC concentration. The two algorithms evaluated in this study were slightly different, with one designed to more closely correspond with the curve of the 'liking' question and the other with the 'wanting more' question on the DEQ, indicating that it is potentially possible to develop algorithms that more subtly differentiate different components of the cannabis effect experience. The algorithms are based on a classifier that uses a large number of EEG features, including the alpha band features identified by Lukas et al. [22] that appear during short bursts of euphoria. By using a classifier trained using artificial intelligence algorithms, this study shows a novel method of identifying PEs in short EEG segments and provides a new theoretical framework for studying cannabis PEs. With these data we suggest that the PE effects are not continuous but rather fluctuate in discrete bursts. For example, a PE level of 70% indicates that 14 out of 20 EEG segments were classified as 'abnormal' with the other 7 being 'normal'. Typically the two types of segments appear interspersed, which we speculate provides evidence for the idea that psychoactive effects wax and wane in bursts on the level of seconds. Furthermore, the absolute level of one or two discrete features is not sufficient to classify a segment as abnormal; rather a complex conjunction of many quantitative EEG features, identified by a machine learning algorithm, is required to accurately classify EEG segments. The exact nature of the conjunction(s) of features that are required to classify a segment as 'abnormal' is beyond the scope of the current publication; we hope to develop insights into this area and divulge them in future publications.

Subjective psychoactive effects of acute cannabis intake include both positive effects, such as relaxation, sociability, creativity, feeling energetic and increased sex drive, and negative effects, including laziness, drowsiness, inability to concentrate, dizziness, nausea, loss of control, anxiety/paranoia and hallucinations [29–32]. Both sedative and stimulative effects are reported in these and other studies [33]. Frequency of positively evaluated effects was shown to decrease over 5–6 years of use [34, 35], indicating an interaction between cannabis effects and cannabis use profile. Tolerance to the stimulative effects has been reported over a 16-day period [36]. In double-blinded placebo-controlled studies, self-report of intoxication level has been shown to have reliably similar dose-response functions to physiological and pharmacokinetic effects [37, 38] suggesting that these subjective reports have value in indicating

Table 3 Relationship between Cognalyzer® version 1 psychoactive effect (PE) levels (log transformed) and the modified Drug Effects Questionnaire (DEQ) scores in the PP population (n = 41)

Drug Effects Questionnaire vs. Cognalyzer® V1 PE levels									
Variables	**DEQ question 1—feel**			**DEQ question 2—high**			**DEQ question 3—dislike**		
	Coefficient	**Standard error**	***p* value**	**Coefficient**	**Standard error**	***p* value**	**Coefficient**	**Standard error**	***p* value**
(Intercept)	9.11	1.15	< 0.001	9.06	1.19	< 0.001	2.5	1.48	0.093
Cognalyzer® V1 THC level	0.38	0.23	0.102	0.26	0.22	0.25	0.38	0.27	0.167
Time 0.5 h	− 0.64	0.32	0.045	− 0.6	0.31	0.052	− 0.54	0.38	0.156
Time 1 h	− 1.15	0.36	0.002	− 1.24	0.35	< 0.001	− 0.4	0.43	0.357
Time 1.5 h	− 2.57	0.34	< 0.001	− 2.98	0.33	< 0.001	− 0.77	0.4	0.058
Time 2 h	− 3.82	0.36	< 0.001	− 4.14	0.35	< 0.001	− 0.77	0.43	0.077
Time 2.5 h	− 4.56	0.37	< 0.001	− 4.85	0.36	< 0.001	− 1.04	0.44	0.018
Time 3 h	− 5.38	0.37	< 0.001	− 5.58	0.35	< 0.001	− 1.37	0.43	0.002
Time 3.5 h	− 6.35	0.39	< 0.001	− 6.65	0.37	< 0.001	− 1.25	0.46	0.007
Time 4 h	− 7.03	0.4	< 0.001	− 7.05	0.39	< 0.001	− 1.86	0.48	< 0.001
Age	− 0.05	0.03	0.153	− 0.05	0.04	0.146	0	0.04	0.976
Gender-male	0.09	0.48	0.851	0.14	0.49	0.779	− 0.64	0.62	0.309
Cognalyzer® PE level:time 0.5 h	− 0.23	0.3	0.453	0.03	0.29	0.914	− 0.67	0.36	0.063
Cognalyzer® PE level:time 1 h	0.24	0.34	0.473	0.31	0.33	0.349	0.07	0.4	0.863
Cognalyzer® PE level:time 1.5 h	− 0.25	0.27	0.356	− 0.31	0.26	0.236	− 0.37	0.32	0.257
Cognalyzer® PE level:time 2 h	− 0.53	0.29	0.068	− 0.53	0.28	0.06	− 0.44	0.34	0.203
Cognalyzer® PE level:time 2.5 h	− 0.46	0.29	0.114	− 0.43	0.28	0.127	− 0.67	0.35	0.054
Cognalyzer® PE level:time 3 h	− 0.45	0.31	0.147	− 0.42	0.3	0.162	− 0.74	0.37	0.046
Cognalyzer® PE level:time 3.5 h	− 0.78	0.33	0.019	− 0.71	0.32	0.027	− 0.72	0.39	0.069
Cognalyzer® PE level:time 4 h	− 0.78	0.3	0.009	− 0.63	0.29	0.028	− 0.72	0.35	0.043

Table 3 continued

Drug Effects Questionnaire vs. Cognalyzer® V1 PE levels						
Variables	**DEQ question 4—like**			**DEQ question 5—more of drug**		
	Coefficient	**Standard error**	***p* value**	**Coefficient**	**Standard error**	***p* value**
(Intercept)	6.94	1.66	< 0.001	1.46	1.77	0.411
Cognalyzer® V1 THC level	0.95	0.35	0.007	0.06	0.3	0.831
Time 0.5 h	− 0.23	0.48	0.64	− 0.05	0.41	0.913
Time 1 h	− 0.53	0.54	0.332	0.18	0.47	0.701
Time 1.5 h	− 1.2	0.51	0.021	− 0.31	0.44	0.479
Time 2 h	− 2.08	0.55	< 0.001	− 0.63	0.47	0.185
Time 2.5 h	− 2.8	0.56	< 0.001	− 0.72	0.48	0.134
Time 3 h	− 3.48	0.55	< 0.001	− 0.48	0.47	0.316
Time 3.5 h	− 4.76	0.59	< 0.001	− 0.46	0.5	0.366
Time 4 h	− 5.37	0.61	< 0.001	− 1.1	0.52	0.036
Age	− 0.01	0.05	0.841	− 0.01	0.05	0.876
Gender-male	1.12	0.69	0.114	1.47	0.74	0.055
Cognalyzer® PE level:time 0.5 h	− 0.77	0.46	0.094	− 0.23	0.39	0.56
Cognalyzer® PE level:time 1 h	− 0.52	0.51	0.31	0.33	0.44	0.451
Cognalyzer® PE level:time 1.5 h	− 0.75	0.41	0.071	− 0.13	0.35	0.705
Cognalyzer® PE level:time 2 h	− 1.26	0.44	0.004	− 0.22	0.38	0.562
Cognalyzer® PE level:time 2.5 h	− 1.13	0.44	0.011	− 0.53	0.38	0.162
Cognalyzer® PE level:time 3 h	− 1.06	0.47	0.025	− 0.34	0.4	0.392
Cognalyzer® PE level:time 3.5 h	− 1.89	0.5	< 0.001	− 0.3	0.43	0.49
Cognalyzer® PE level:time 4 h	− 1.77	0.45	< 0.001	− 0.63	0.39	0.104

Table 4 Relationship between Cognalyzer® version 2 psychoactive effect (PE) levels (log transformed) and the modified Drug Effects Questionnaire (DEQ) scores in the PP population (n = 41)

Drug Effects Questionnaire vs. Cognalyzer® V2 PE levels									
Variables	**DEQ question 1—feel**			**DEQ question 2—high**			**DEQ question 3—dislike**		
	Coefficient	**Standard error**	***p* value**	**Coefficient**	**Standard error**	***p* value**	**Coefficient**	**Standard error**	***p* value**
(Intercept)	9.25	1.11	< 0.001	9.15	1.12	< 0.001	2.32	1.36	0.09
Cognalyzer® V2 THC level	0.02	0.32	0.954	0.06	0.33	0.849	0.23	0.41	0.568
Time 0.5 h	− 0.65	0.29	0.028	− 0.69	0.3	0.022	− 0.34	0.37	0.357
Time 1 h	− 1.17	0.31	< 0.001	− 1.34	0.31	< 0.001	− 0.45	0.39	0.251
Time 1.5 h	− 2.39	0.31	< 0.001	− 2.87	0.32	< 0.001	− 0.65	0.39	0.097
Time 2 h	− 3.6	0.32	< 0.001	− 3.98	0.32	< 0.001	− 0.79	0.4	0.049
Time 2.5 h	− 4.45	0.33	< 0.001	− 4.77	0.34	< 0.001	− 0.8	0.42	0.059
Time 3 h	− 5.27	0.33	< 0.001	− 5.45	0.34	< 0.001	− 1.29	0.42	0.002
Time 3.5 h	− 6.13	0.34	< 0.001	− 6.37	0.35	< 0.001	− 1.33	0.43	0.002
Time 4 h	− 6.6	0.35	< 0.001	− 6.75	0.36	< 0.001	− 1.65	0.44	< 0.001
Age	− 0.06	0.03	0.086	− 0.06	0.03	0.089	0	0.04	0.92
Gender-male	0.18	0.47	0.696	0.22	0.47	0.642	− 0.62	0.57	0.287
Cognalyzer® PE level:time 0.5 h	− 0.26	0.41	0.527	− 0.28	0.42	0.505	− 0.06	0.52	0.914
Cognalyzer® PE level:time 1 h	0.5	0.37	0.184	0.33	0.38	0.391	0.14	0.47	0.759
Cognalyzer® PE level:time 1.5 h	− 0.18	0.39	0.64	− 0.33	0.4	0.402	− 0.11	0.49	0.814
Cognalyzer® PE level:time 2 h	− 0.26	0.36	0.477	− 0.41	0.37	0.263	− 0.33	0.46	0.464
Cognalyzer® PE level:time 2.5 h	− 0.15	0.37	0.697	− 0.3	0.38	0.432	− 0.34	0.47	0.472
Cognalyzer® PE level:time 3 h	0.04	0.38	0.916	0.02	0.39	0.96	− 0.51	0.48	0.283
Cognalyzer® PE level:time 3.5 h	− 0.26	0.37	0.481	− 0.32	0.38	0.404	− 0.65	0.47	0.165
Cognalyzer® PE level:time 4 h	− 0.08	0.35	0.814	− 0.17	0.36	0.634	− 0.33	0.45	0.459

Table 4 continued

Drug Effects Questionnaire vs. Cognalyzer® V2 PE levels						
Variables	DEQ question 4—like			DEQ question 5—more of drug		
	Coefficient	Standard error	*p* value	Coefficient	Standard error	*p* value
(Intercept)	6.46	1.67	< 0.001	0.81	1.85	0.661
Cognalyzer® V2 THC level	− 0.21	0.51	0.687	− 1.41	0.43	0.001
Time 0.5 h	− 0.13	0.47	0.774	0.33	0.39	0.398
Time 1 h	− 0.45	0.49	0.359	0.2	0.41	0.623
Time 1.5 h	− 0.98	0.49	0.048	0.01	0.41	0.976
Time 2 h	− 1.56	0.5	0.002	− 0.25	0.42	0.552
Time 2.5 h	− 2.48	0.53	< 0.001	− 0.24	0.45	0.593
Time 3 h	− 3.39	0.52	< 0.001	− 0.02	0.44	0.964
Time 3.5 h	− 4.04	0.54	< 0.001	− 0.25	0.45	0.586
Time 4 h	− 4.77	0.55	< 0.001	− 0.76	0.46	0.103
Age	− 0.01	0.05	0.874	0	0.06	0.969
Gender-male	1.11	0.7	0.123	1.37	0.78	0.087
Cognalyzer® PE level:time 0.5 h	− 0.28	0.66	0.665	1.52	0.56	0.007
Cognalyzer® PE level:time 1 h	0.32	0.59	0.585	1.5	0.5	0.003
Cognalyzer® PE level:time 1.5 h	0.26	0.61	0.672	1.43	0.52	0.007
Cognalyzer® PE level:time 2 h	0.1	0.57	0.86	1.34	0.49	0.006
Cognalyzer® PE level:time 2.5 h	− 0.04	0.59	0.947	1.15	0.5	0.023
Cognalyzer® PE level:time 3 h	− 0.24	0.6	0.69	1.08	0.51	0.034
Cognalyzer® PE level:time 3.5 h	− 0.35	0.59	0.556	0.96	0.5	0.055
Cognalyzer® PE level:time 4 h	− 0.32	0.56	0.572	0.99	0.48	0.038

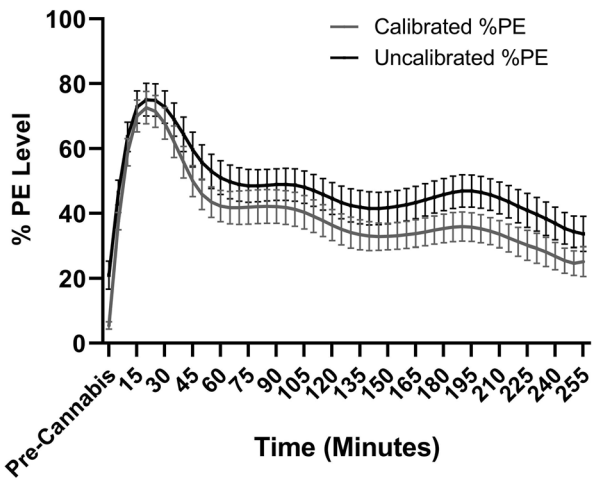


Fig. 2 Mean % psychoactive effect (PE) predictions with and without application of the calibration method designed to account for the varying psychoactive effect sensitivities between the participants. Error bars indicate ± 1 SEM

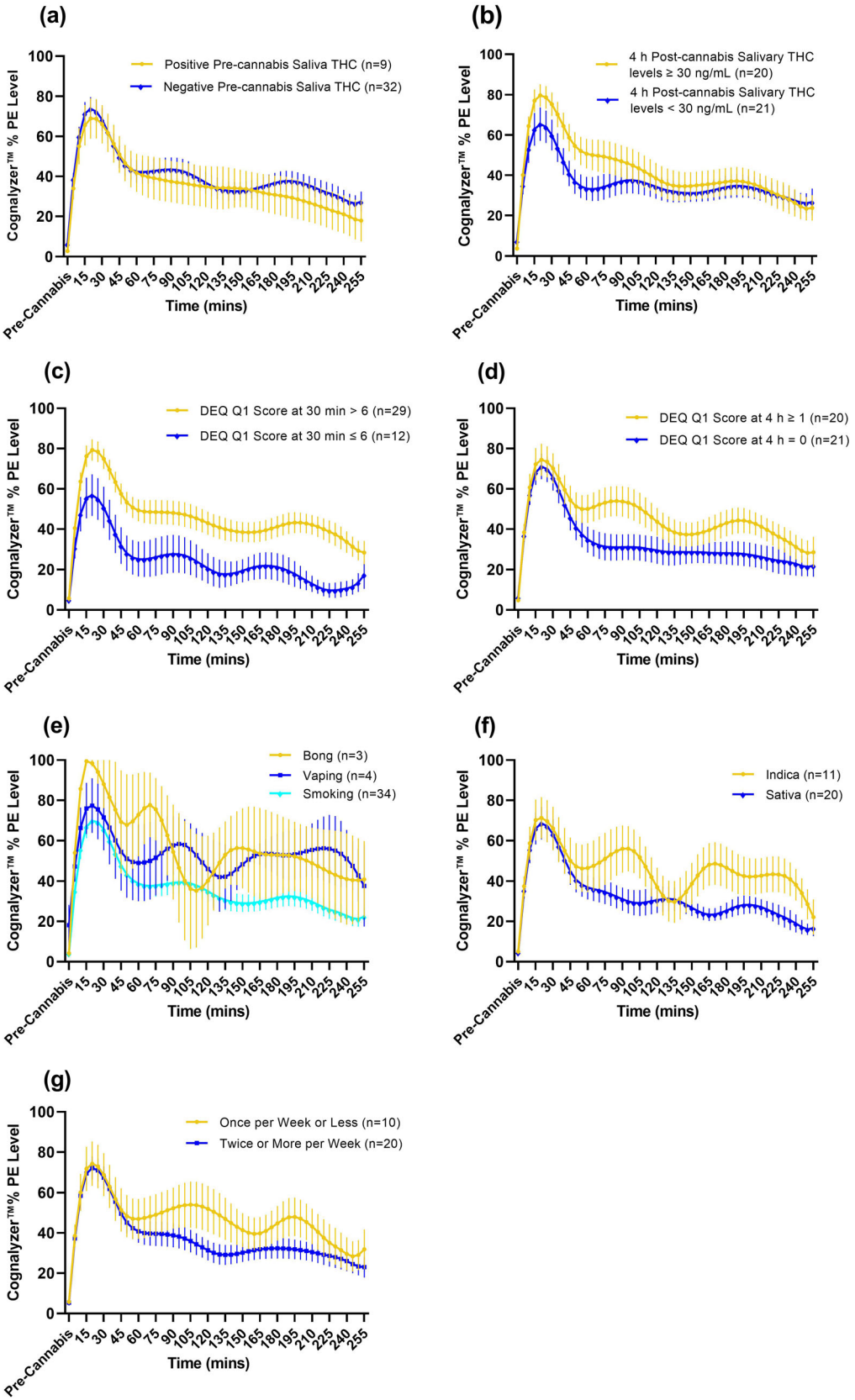
the objective intensity of the psychoactive effects. Little appears to be known about the exact temporal relationship between these acute subjective effects and cannabis intake and or how the intake method influences these factors. Acute cannabis intake may also produce measurable impairment in cognition and psychomotor function, but there is significant variability with dose, route of administration and individual cannabis tolerance [7, 39–41]. The cannabinoid and terpene profiles also have some influence on these subjective ratings [42, 43].

The improvement in correspondence between PE and subjectively reported effects after 'calibration' of the algorithm has interesting implications. Some participants present with a large number of false-positive segments in the pre-inhalation test, implying that their EEGs before cannabis inhalation are more similar to 'abnormal' EEGs. It may be that these participants had continuing alterations in their EEG patterns due to frequent cannabis intake. It is known that frequent cannabis intake alters the PEs, as shown by decrease in pleasurable drug effects, indicating a level of tolerance [34–36]. Participants in this study had variable cannabis use histories. The analysis of the

Fig. 3 Cognalyzer® THC Psychoactive Effect (PE) (method 1) in the per-protocol (PP) population subgroups based on **a** pre-inhalation oral fluid THC test; **b** 4-h post-inhalation oral fluid THC test; **c** Drug Effects Questionnaire (DEQ) Q1: "Feeling high" score of > 6 and ≤ 6 at 30 min; **d** DEQ Q1: "Feeling high" score of ≥ 1 or $= 0$ at 4 h; **e** cannabis inhalation method; **f** indica vs. sativa; **g** weekly reported frequency of cannabis use. Values presented as mean ± standard error of the mean

subgroups identified by cannabis use frequency (Fig. 3g) showed that the results were not significantly different between the frequency groups; however, the results were orderly and indicate promise in the ability of the algorithm to differentiate how cannabis effects might differ between more and less frequent users. Although the peak magnitude of the PE did not appear to differ between groups, in the several hours after the peak PE effect the lowest frequency users had the highest measured PE levels, and the highest frequency users the lowest measured PE levels, with the intermediate group falling between at every time point. Further study with groups of frequent vs. infrequent cannabis users would be useful to develop this concept.

In our analysis of the two groups categorized by their self-reported DEQ score at 30-min post-inhalation, we showed that the magnitude of the PE score was sensitive to the level of the subjective self-reported drug effect. The PE level of participants reporting a greater DEQ was higher at every time point across the 4-h measurement period, and this effect was significant at all time points from 30 to 120 min. When participants were grouped instead by their DEQ score at the final (4 h) time point, the PE levels between groups were no longer significantly different although descriptively the effect looked the same, with PE level being higher in the group reporting higher DEQ scores at every time point. Similarly, when participants were grouped by the concentration of THC detected in the oral fluid sample at 4 h post-inhalation, the PE scores also showed significant differences between the groups. The participants with higher oral fluid levels had greater peak PE magnitudes and overall higher PE levels across


(a)
Positive Pre-cannabis Saliva THC (n=9)
Negative Pre-cannabis Saliva THC (n=32)
(b)
4 h Post-cannabis Salivary THC levels ≥ 30 ng/mL (n=20)
4 h Post-cannabis Salivary THC levels < 30 ng/mL (n=21)
(c)
DEQ Q1 Score at 30 min > 6 (n=29)
DEQ Q1 Score at 30 min ≤ 6 (n=12)
(d)
DEQ Q1 Score at 4 h ≥ 1 (n=20)
DEQ Q1 Score at 4 h = 0 (n=21)
(e)
Bong (n=3)
Vaping (n=4)
Smoking (n=34)
(f)
Indica (n=11)
Sativa (n=20)
(g)
Once per Week or Less (n=10)
Twice or More per Week (n=20)
Cognalyzer™ % PE Level
Time (mins)
Pre-Cannabis
0 20 40 60 80 100
15 30 45 60 75 90 105 120 135 150 165 180 195 210 225 240 255

the entire test duration—this may be because these participants inhaled more cannabis ad libitum. This effect did not occur for participants grouped by their initial oral fluid level; we suggest that in most cases a high initial oral fluid level did not indicate that the participant was currently experiencing cannabis effects but rather these test results were indicative of residual THC levels in more chronic users [44]. We also acknowledge that the 3 days of abstinence required from our participants was only self-reported; it is certainly possible that some participants failed to adhere to this requirement. Participants with low pre-cannabis oral fluid THC concentration had significant increases in PE for all time points and those with high pre-cannabis concentrations resolved their PE at 3.5 h. We hypothesize that those who had high pre-inhalation oral fluid THC concentration may have been more frequent smokers, and this result could have occurred because of developed THC tolerance. This aligns with the results of Fig. 3g. Taken together, these results indicate that the Cognalyzer® algorithm is able to provide an objective quantification of the magnitude of the psychoactive effect of cannabis, a result that has not previously been achieved. Other EEG methods for detecting cannabis effects in EEG, for example, quantifying decreases in P300 amplitude [45], have not demonstrated their efficacy at quantifying the levels of PE over time and require the active involvement of the participant.

The differences observed between strains, while not significant, indicate that the measured PE might be sensitive to the 'entourage effects' of differing cannabinoid and terpene profiles [43]. In so far as a potential strain difference might exist, it might potentially be due to differences between participants who select strains that advertise particular types of effects—for example, 'indica' strains are widely thought to be more sedating than sativa strains, even though their terpene analyses show no significant differences between strains [46]. The observational nature of the study prevents us from drawing any conclusions. Similarly, although with only four participants the vaping result is not statistically significant, we note that the pattern of higher PE for vaping vs. smoking is consistent with the literature showing that vaping produces higher blood concentrations of THC at the peak [8].

There are some noteworthy limitations of the current study that need further investigation. First, the study lacked positive and negative control conditions because of the observational nature of the experimental design. This limits the ability to definitively claim that the changes in the Cognalyzer™ algorithm are solely due to the psychoactive effects of cannabis. This should be rectified in a more rigorously controlled experimental study, which could also include more control over the cannabis dosage and intake method. However, the adequate performance of the algorithm over such a wide range of dosing regimens is promising. Second, the current study does not report objective measures of actual impairment caused by psychoactive effects. Further studies that include both the PE level and impairment tests on a realistic task, such as a driving simulator, would allow for relationships between the two measures to be quantified and provide evidence to make the algorithm useful for impairment detection in law enforcement and employee drug testing applications.

CONCLUSION

The ability of the Cognalyzer® algorithm to objectively quantify strength and determine the time course of the psychoactive effects of cannabis products (a measure we would like to refer to as 'psychoactivity', to distinguish it from other more conventional pharmacodynamic effects) has the potential to allow it to be used in several interesting applications. By providing an objective standard for comparing products and doses, it could be used to provide product effect information for existing medical and recreational products. No such standard exists, and licensed producers are forced to rely on unreliable subjective information and in some cases crowd-sourced product reviews when making R&D and product innovation decisions. Both producers and consumers are interested in learning with some degree of certainty details about the effects that consumption of a given

product might induce, both in terms of simple effects like onset time, duration, and potency and in terms of more complex psychographic parameters like mood changes. A programme of consumer product research incorporating the PE level measurements from the Cognalyzer® would allow consumers to compare effect curves created by different products at different dosages or modes of consumption (e.g., vaping) and has future applications for not only inhaled cannabis but also edibles, beverages, sublingual doses, etc. By further relating effect curves to different populations, such as frequent vs. infrequent consumers, personality traits or demographic characteristics like BMI or sex, consumers could gain valuable insight into how to use cannabis products safely and efficaciously. Heavy users may require significantly higher doses to achieve the desired potency of effect—this could be quantified. For medical applications like pain management where psychoactive effects are sometimes undesirable, the therapeutic effects could be related to psychoactive effect curves. Enabling a new method of objective cannabis product effect descriptions would benefit both the cannabis industry and society as a whole by allowing concrete objective statements about specific product effects to be claimed. The non-invasive nature of the Cognalyzer® measurement would allow this information to be developed within a consumer research framework. It could also be utilized within a clinical trial framework to support therapeutic claims or to study the pharmacodynamics of cannabinoid formulations or delivery systems, such as nano-emulsifications. For safety and law enforcement purposes, the Cognalyzer® measurement opens up for the first time the possibility to further study the relationship between the standard Cognalyzer® cannabis psychoactivity and cognitive performance while driving or conducting safety-sensitive or safety-critical tasks and find a Cognalyzer PE threshold to determine when it is safe or not to drive or work.

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Compliance with Ethics Guidelines. The trial received research ethics board approval on 14 February 2020 from the Institutional Review Board (IRB) Services, Aurora, Ontario (Pro00041616). All participants provided written informed consent prior to initiation of study procedures. The study was conducted in compliance with the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) Guideline for Good Clinical Practice (GCP) and in accordance with the Declaration of Helsinki guidelines and its subsequent amendments. The study was performed in accordance with the Helsinki Declaration of 1964 and its later amendments.

Data Availability. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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