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Pupillary dynamics as a marker of acute cannabis inhalation

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ABSTRACT

Introduction: Acute cannabis use has been found to affect pupil size and pupillary dynamics. Law enforcement may consider ocular changes in their examinations to determine drug impairment and the source, including from cannabis. A limited number of studies have used pupillometer technology to provide an objective measure of pupillary changes associated with cannabis use. The purpose of the study was to examine the sensitivity and specificity of pupil size and dynamics, measured with a pupillometer, associated with recent cannabis inhalation.

Methods: Participants ($n = 126$) completed a pupillometer assessment, using the NeurOptics PLR-3000 at three times. Of the 126 participants, 95 completed assessments at baseline, and at 40 min and 100 min following 15 min of *ad libitum* inhalation of self-provided cannabis flower or concentrate products. Thirty-one participants completed the same assessments without using cannabis. Sensitivity, specificity and accuracy were calculated for pupil size and dynamics measures associated with recent cannabis use versus no use, for both post-use time points. Least absolute shrinkage and selection operator models were used to identify the combination of ocular metrics that were most predictive and parsimonious.

Results: Following cannabis use, the pupillary measure with the highest area under the curve was percent change in pupil size, which decreased after cannabis use, with an area under the curve of 0.73 at 40 min and 0.75 at 100 min following cannabis use. Considering variables together in a least absolute shrinkage and selection operator model did not meaningfully improve prediction over individual measures.

Discussion: Consistent with some prior studies, we did not find that cannabis use was associated with substantial and consistent change in the maximum pupil size (measured in darkness) relative to controls. However, diminished pupil dynamics, such as constriction in response to light and recovery dilation, were more predictive of recent cannabis use, consistent with limited prior studies.

Conclusions: Pupillary dynamics, when measured with an objective test, may contribute to providing an indication of recent cannabis inhalation.

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Introduction

Current approaches to assessing drug impairment among drivers, such as the standard field sobriety test, have limited accuracy for detecting cannabis related impairment [1–3]. As an added challenge, there is limited utility of blood delta-9-tetrahydrocannabinol concentrations for inferring impairment, or even recent cannabis use [4,5]. As the established approaches for determining alcohol impairment do not translate to cannabis impairment, there is a need for objective tests of the acute central nervous system effects of cannabis, especially approaches that could be deployed in a timely manner at the scene of a transportation crash or incident, in a post-incident review, or in an occupational setting. One promising line of inquiry is pupillary measures, as many drugs acutely affect ocular parameters [6]. Delta-9-tetrahydrocannabinol, the primary psychoactive compound in cannabis, activates cannabinoid

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receptors (particularly cannabinoid receptor 1) in the central nervous system, leading to changes in autonomic function. This activation can result in changes to pupillary response (the iris muscles) due to modulation of sympathetic and parasympathetic pathways. Specially trained law enforcement officers, called drug recognition experts, have long examined pupil size, pupillary dynamics, and ocular movements when assessing the potential for acute drug effect and potential impairment [7,8]. If pupillometer technology could be leveraged to identify pupillary changes associated with acute cannabis use, it could provide a portable, non-invasive, and objective indicator of recent cannabis use that could be deployed in settings such as roadside driver evaluations or occupational investigations [9].

A range of research methods has been employed to study pupillary changes associated with acute cannabis use. For example, a secondary analysis of physician observations of drugged driving suspects found a minority of suspects had dilated pupils at higher blood delta-9-tetrahydrocannabinol concentrations [10]. A study by Hartman et al. [11] used case-control design to study examinations previously conducted by drug recognition experts after a traffic stop. They found that subjectively assessed rebound dilation was present in 70.9% of the cases and 0% of the controls. Furthermore, they found pupils were larger in cases than the controls, in all lighting conditions. However, as a major limitation to these studies, they used subjective, non-blinded observations in the setting of a law enforcement activity, which are susceptible to confirmation and observer bias [10,11]. Studies comparing manual observations of pupil size, either between observers or as compared to automated technology, have demonstrated poor reliability of human assessments [12–14]. Based on this literature, an accurate measure of the pupil would require the use of technology.

Several studies have used pupillometer technology to objectively measure pupil size following acute cannabis smoking. However, there have been inconsistent findings as it relates to pupil size. Some studies on pupil size (pupil diameter) following acute cannabis smoking have resulted in inconsistent findings, with some studies finding increased size post-use [15,16], and others failing to find a consistent effect from cannabis [17], or even that pupil size decreased in some conditions [18,19]. Despite these inconsistent results associated with changes to pupil diameter, there are relatively more consistent findings as it relates to objectively measured pupil dynamics in response to light. A few studies have found that the pupillary light reflex was weakened by cannabis, such that there is a diminished pupil constriction after a light stimulus [20–23]. In summary, among studies with objective measures of pupil size (rather than manual observations), there is somewhat conflicting evidence if acute cannabis results in changes to static pupil diameter, and if the change is to a larger or smaller pupil. However, there are more consistent findings of altered pupillary dynamics, suggesting there may be a diminished pupillary response to light following acuate cannabis use. In this study, we used a research-quality pupillometer to investigate static pupil size and pupil dynamics following naturalistic acute cannabis inhalation.

Methods

Participants

Participants ($n = 126$) were recruited into one of four cannabis use categories based on the frequency of any type of cannabis product use (daily versus occasionally, defined as use on at least one day per month but no more than three days per week over the last three months) and the delta-9-tetrahydrocannabinol concentration of cannabis product used during the study:

1. flower containing 15% to 30% total tetrahydrocannabinol (delta-9-tetrahydrocannabinol + tetrahydrocannbinolic acid); or
2. cannabis extract products, e.g., vape cartridges, 'dabs', resins, hereafter referred to as concentrates.

The groups were as follows:

1. daily cannabis use with the use of flower during the study ($n = 34$);
2. daily cannabis use with use of concentrate product during the study ($n = 32$);
3. occasional cannabis use with use of flower during the study ($n = 29$); and
4. no use of cannabis as a comparison group ($n = 31$).

Participants completed informed consent at a visit before the data collection visit. The Colorado Multiple Institutional Review Board approved study procedures (20-0949).

Data collection

Data collection was conducted at an off-campus research site. Participants were asked to abstain from cannabis use for at least 8 h prior to the start of their visit. At baseline, participants self-reported clinically significant medical history and medications. Participants completed a timeline followback calendar to verify all cannabis products used prior to the data collection

visit, which asked about the prior 30 days for participants in the daily use group or the prior 90 days for the occasional use group.

Pupil diameter and pupillary dynamics were measured with the NeurOptics PLR-3000 pupillometer [24]. The PLR-3000 pupillometer is a handheld instrument that uses infrared videography to quantify pupil diameter. We conducted the assessment in a darkened room to standardize conditions across participants and to ensure both eyes were exposed to the same baseline condition of a darkened room before the light stimulus. Before measurement, participants rested in a darkened room for approximately 2 min. The eye cup of the pupillometer was placed flush with the participant's face. For standardization, the right eye was measured first. The participants were requested to keep their eyes open as wide as possible during the test. The pupillometer recorded pupil diameter during one second of darkness, a 0.8 sec (800 msec) interval of bright light (180 microwatts) and a subsequent 3 sec of recovery. Measures were recorded every 33 msec during the test. The raw data files included pupil diameter and a binary indicator of whether the pupil was obstructed by an involuntarily eyelid blink at each time point. The pupillometer flagged pupil tests in which more than 60% of the attempted measurements were obstructed. In those instances, the data collection test was repeated. The right eye was tested first, followed by the left eye.

After baseline assessments, participants were given up to 15 min to inhale cannabis *ad libitum*. The non-use group was given the choice to relax for an equivalent 15 min, but did not use cannabis. Participants supplied their own cannabis labeled with total tetrahydrocannabinol (delta-9-tetrahydrocannabinol + tetrahydrocannbinolic acid) concentration, from a licensed Colorado dispensary. At baseline (before use) and two times following inhalation, participants were surveyed about their perceived drug effect. A blood sample was collected at baseline and at three times following cannabis use.

Demographic characteristics

Participants were asked about their gender, age, race/ethnicity, and frequency of cannabis use, and research staff measured the participants' height and weight.

Cannabis use

Participants used a range of products including flower buds, pre-rolled joints, cannabis concentrates (e.g. "sugar", "shatter") and oil. Methods for flower consumption included: joints, bongs, "dry herb" vaporizers; for concentrate consumption: dab rigs, "nectar collectors", and vaporizers. The percent total tetrahydrocannabinol concentration of the product was recorded, as was the number of minutes spent inhaling and the number of inhalations (or "hits").

Perceived drug effect

Subjective drug effect was measured with a visual analogue scale on which participants were asked to mark the point on the line indicating "how high you are feeling right now?" ranging from "not high at all" (0 mm) to "most high ever" (100 mm).

Blood cannabinoid concentrations

At baseline and three times following use, a certified phlebotomist collected approximately 3 mL of blood using standard sterile phlebotomy techniques into grey-top tubes containing 100 mg sodium fluoride and 20 mg potassium oxalate additive and stored at approximately 4 °C for analysis within 30 days. Whole blood samples were transported on dry ice and cold packs for analysis.

Pupillary measures

Post-cannabis use pupillary measurements were obtained at approximately 40 min and 100 min following the start of cannabis inhalation. From the trajectories of pupil diameter, and first and second derivatives of the trajectories, eight scalar measures were calculated.

(1) *Maximum pupil diameter* (mm) was the average pupil diameter recorded by the device for half a second before the light stimulus.
(2) *Minimum pupil diameter* (mm) was the smallest pupil diameter following the light stimulus.
(3) The *percent change in pupil size* was calculated from the maximum to minimum pupil diameter (percent).
(4) *Pupil latency* (sec) was defined as the time to onset of constriction following a light stimulus.
(5) *Pupil constriction velocity* was calculated from the start of the test to the time of minimum pupil diameter (mm/sec). Constriction velocity was calculated as the difference between the maximum diameter and the minimum diameter divided by the time of the minimum constriction −1 (time of light stimulus).
(6) *Maximum pupil constriction velocity* (mm/sec) was calculated as the difference between each pair of consecutive measurement points divided by

the time between them, from the time of the light stimulus to the time to reach the minimum diameter and then finding the maximum.

(7) *Recovery dilation velocity* was calculated from the time of minimum pupil diameter to the end of the test, divided by the time elapsed between those points (mm/sec).

(8) *Recovery dilation* area under the curve (AUC) was calculated as the area between the pupil trajectory and the baseline value after the minimum diameter is reached. Thus, a larger AUC value would indicate greater recovery of dilation pupil dynamics.

We examined data from both eyes separately and found similar results. Given this, we had the option of reporting results from the eyes separately or combining the data from both eyes in one model. For simplicity, we used the trajectories from the right eye for analysis. Because the observations between eyes are highly correlated, we would not expect results to substantially differ if the left eye was analyzed instead. In cases for which right eye data were not usable ($n=3$ at Post 1; $n=1$ at Post 2), the left eye trajectory was used in its place, given the similarity between the two eyes. When multiple tests were conducted for an eye at the same time point, the best trajectory was selected based on the least number of extreme oscillations, longer test duration, least missing data points, and trajectory appearance on visual inspection. Trajectories were smoothed with a nonlinear regression on the 50th percentile of data points.

Statistical analysis

First, we started by considering baseline measurements. We present these descriptive results by the four participant groups, consistent with the overall study design and approach. Separate ANOVA models were used to test differences in these variables among the three subject groups at the baseline (pre-consumption) assessment to ensure there were no systematic group differences at baseline that could be a confounder. Subsequently, our approach to the analysis was to consider what information might be available in a forensic context, which would only be observations post-cannabis use. Thus, although we collected measures at multiple time points per participant, we did not treat the data as repeated measures, including the baseline measurements. Separate logistic regression models were used to assess the ability to discriminate between recent cannabis use ($y=1$) and no use ($y=0$; i.e., data from control subjects) for each scalar variable at each of the post-consumption assessments (not including the pre-consumption assessment). Participants in the daily-concentrate, daily-flower and occasional-flower use groups were categorized as "recent cannabis use", and participants in the no use group were categorized as "no recent cannabis use".

Receiver operator characteristic (ROC) curves were created for each model, and sensitivity, specificity, accuracy and the AUC were calculated. Two separate logistic regression least absolute shrinkage and selection operator (LASSO) models, one for each post-consumption assessment (separately), were used to create a model with a combination of the scalar pupil variables. LASSO is a variable selection model that imposes a penalty to preference the most parsimonious model. Logistic LASSO assumes a linear relationship between the log-odds of the outcome and the predictors and performs variable selection while addressing multicollinearity. While logistic LASSO can mitigate multicollinearity by shrinking correlated predictors toward zero, it does not eliminate collinearity entirely. Variables with the strongest associations remain in the model, while variables with weak associations are removed. For the LASSO models, we used 10-fold cross-validation and chose the penalty λ (lambda) that minimized the binomial deviance to define the final model.

Lastly, we conducted an exploratory analysis to consider the relationship between blood delta-9-tetrahydrocannabinol concentrations and the analytic variables used in the LASSO model.

Results

Participant characteristics

Table 1 presents demographic characteristics of the participants ($n=126$). Participants were mostly white (81%), 57.9% identified their gender as male, 40.5% as female, 1.6% as non-binary. Most (73.0%) participants were between 25 years and 34 years old.

Cannabis use and perceived drug effect

Table 2 presents group-level information about cannabis use during the study visit. The average total tetrahydrocannabinol concentration by weight according to the product package label was 20.8% and 22.3% for flower products (occasional and daily use groups, respectively) and 75.8% for concentrate products. Participants smoked for an average of 7.6 min, and an average of 12 inhalations. Across all cannabis-use participants, the

self-reported drug effect (on a visual analog scale of 0 mm to 100 mm) was an average of 0.4 mm at baseline, 77.3 mm at post-use time 1 and 46.6 mm at post-use time 2 (data not shown). Baseline whole blood tetrahydrocannabinol concentrations were related to subjects' cannabis use history in that delta-9-tetrahydrocannabinol concentrations were 2.6 µg/L and 6.0 µg/L for the daily use groups and 0.3 µg/L for the occasional-flower group. The pupillometer assessments were conducted at an average of 40 min (Post time 1) and 98 min (Post time 2) following cannabis use.

Table 1. Participant characteristics, overall and by use group ($n = 126$).

	No use ($n = 31$)	Occasional use – inhaled flower ($n = 29$)	Daily use – inhaled flower ($n = 34$)	Daily use – inhaled concentrates ($n = 32$)
Gender				
Female, *n* (%)	61.3 (19)	34.5 (10)	35.3 (12)	31.3 (10)
Male, *n* (%)	38.7 (12)	65.5 (19)	58.8 (20)	68.8 (22)
Non-binary, *n* (%)	0	0	5.9 (2)	0
Age (years)				
25–34, *n* (%)	64.5 (20)	72.4 (21)	70.6 (24)	84.4 (27)
35–44, *n* (%)	25.8 (8)	24.1 (7)	26.5 (9)	15.6 (5)
45–55, *n* (%)	9.7 (3)	3.5 (1)	2.9 (1)	0
Race/ethnicity				
Black/African American, *n* (%)	6.5 (2)	10.3 (3)	17.7 (6)	6.25 (2)
Hispanic, *n* (%)	12.9 (4)	13.8 (4)	14.7 (5)	9.4 (3)
Non-Hispanic, White, *n* (%)	74.2 (23)	69.0 (20)	67.7 (23)	81.3 (26)
Other, *n* (%)	6.5 (2)	6.9 (2)	0	3.1 (1)
Body mass index (kg/m^2), median (IQR)	23.9 (22.2–27.4)	23.9 (22.0–27.9)	24.9 (22.4–27.3)	26.5 (23.7–29.5)

Baseline pupillometry measurements

First, we examined the baseline values between the four groups, using ANOVAs, and found no significant differences across the four groups in any baseline values (See Supplemental Table 1 for *P* values).

Consistency of pupillometry measurements

Next, we examined the pupillometry results of the participants who did not use cannabis but completed the pupillometer assessment at three time points. The purpose of this analysis was to examine the unlikely possibility that there could be habituation to the pupillary light reflex with repeated administrations. Additionally, the purpose was to examine the variability in an individual's response over time. Among the control group, the values were consistent across the three timepoints, suggesting that the individual pupil response was stable across time, and multiple episodes of pupillometry did not influence the measurements obtained.

Post-cannabis use

Table 3 presents the means and standard deviations of the pupillary measures at the two post-cannabis use time points, by each of the participant groups. We present descriptive results by participant group to consider the possibility of different pupillary responses associated with the frequency of cannabis use. At the first post-use assessment, the average starting pupil diameter was 6.07 mm for the non-use group, 6.00 mm for the occasional use group, 5.90 mm for the daily

Table 2. Cannabis use, self-reported drug effect and blood tetrahydrocannabinol concentration, and timing of pupillometry, overall and by participant group.

Cannabis use	Occasional use - inhaled flower ($n = 29$) Median (IQR)	Daily use - inhaled flower ($n = 34$) Median (IQR)	Daily use - inhaled concentrates ($n = 32$) Median (IQR)
Total tetrahydrocannabinol concentration*, in percent, by weight (per product package labeling)	21.82 (15.73–23.50)	75.52 (69.93–80.60)	22.50 (19.70–25.07)
Minutes inhaling	6.00 (4.00–10.00)	7.00 (5.00–11.50)	7.00 (4.00–12.00)
Number of inhalations (hits)	11 (7–14)	6 (3–11)	11 (7–18)
Visual analog scale: feel high (0 to 100 mm)			
Baseline	0 (0–0)	0.00 (0.00–0.06)	0.00 (0.00–0.50)
Post use time 1 (mean of 3 min post use)	79.00 (74.75–90.00)	76.15 (68.00–82.31)	80.75 (73.00–87.00)
Post use time 2 (mean of 100 min post use)	62.00 (42.75–69.00)	30.25 (21.50–50.63)	49.13 (27.00–63.00)
Whole blood tetrahydrocannabinol concentration (µg/L)			
Baseline	0 (0–0)	2.88 (0.62–6.76)	1.15 (0.55–3.00)
Post 1 (mean of 13 min post use)	8.00 (5.88–17.96)	42.14 (15.91–97.89)	27.47 (16.54–54.75)
Post 2 (mean of 54 min post use)	2.88 (2.14–9.73)	16.30 (7.51–31.97)	10.35 (6.88–20.71)
Post 3 (mean of 113 min post use)	1.64 (1.13–4.27)	9.83 (4.32–19.98)	5.57 (3.00–10.82)
Timing of pupillometry measurements			
Post 1 (mean 40.3 min)	40.00 (37.00-44.00)	40.00 (37.50-45.00)	39.00 (37.00–42.00)
Post 2 (mean 97.8 min)	99.00 (96.00-101.00)	97.00 (93.50-100.00)	95.50 (93.00–101.00)

Note. All measures of time are from the initial inhalation of cannabis.
*Total tetrahydrocannabinol (delta-9-tetrahydrocannabinol + tetrahydrocannbinolic acid) concentration

Table 3. Pupillary measures by use group at the two post-use assessments.

	No-use Median (IQR)		Occasional use-inhaled flower Median (IQR)		Daily use-inhaled flower Median (IQR)		Daily use-inhaled concentrates Median (IQR)	
	Post 1 assessment*	Post 2 assessment	Post 1 assessment	Post 2 assessment	Post 1 assessment	Post 2 assessment	Post 1 assessment	Post 2 assessment
Maximum pupil diameter (mm)	6.15 (5.37–6.78)	6.09 (5.56–6.67)	6.25 (5.88–6.36)	6.35 (5.75–6.58)	5.87 (5.25–6.63)	6.12 (5.59–6.77)	6.00 (5.66–6.45)	6.23 (5.91–6.88)
Minimum pupil diameter after illumination (mm)	3.34 (3.01–3.98)	3.36 (3.15–3.68)	3.64 (3.42–4.03)	3.81 (3.50–4.22)	3.80 (3.02–4.39)	4.00 (3.22–4.36)	3.53 (3.20–3.97)	3.66 (3.39–4.29)
Percent change pupil size (%)	−44.07 (-46.50 – -39.73)	−43.94 (-47.23 – -41.20)	−40.11 (-43.03 – -35.12)	−39.22 (-42.51 – -33.50)	−36.31 (-42.21 – -31.06)	−38.49 (-42.25 – -33.02)	−39.03 (-44.72 – -36.03)	−38.83 (-43.63 – -34.60)
Pupil latency (sec)	0.10 (0.07–0.14)	0.10 (0.04–0.14)	0.10 (0.04–0.14)	0.10 (0.07–0.14)	0.14 (0.07–0.17)	0.10 (0.07–0.14)	0.10 (0.02–0.14)	0.14 (0.09–0.14)
Pupil constriction velocity (mm/sec)	−2.15 (-2.36, -1.79)	−2.08 (-2.38 – -1.92)	−2.03 (-2.21 – -1.70)	−2.02 (-2.23 – -1.62)	−1.79 (-2.06 – -1.59)	−1.92 (-2.12 – -1.75)	−1.96 (-2.19 – -1.79)	−2.02 (-2.28 – -1.87)
Pupil max constriction velocity (mm/sec)	−4.27 (-4.78 – -3.87)	−4.37 (-4.99 – -3.55)	−4.09 (-4.68 – -3.44)	−4.30 (-4.75 – -3.36)	−3.92 (-4.31, -3.28)	−3.84 (-4.37 – -3.45)	−3.85 (-4.61 – -3.44)	−4.37 (-4.66 – -3.87)
Recovery dilation velocity (mm/sec)	0.53 (0.47–0.60)	0.53 (0.45–0.61)	0.48 (0.46–0.56)	0.54 (0.46–0.63)	0.47 (0.36–0.56)	0.49 (0.42–0.58)	0.53 (0.38–0.61)	0.54 (0.44–0.62)
Recovery dilation area under the curve (mm x sec)	4.72 (3.73–5.44)	4.37 (3.81–5.32)	3.87 (3.12–4.71)	3.41 (3.15–4.62)	3.57 (3.01–4.32)	3.56 (3.18–4.46)	4.10 (3.31–4.69)	4.17 (3.48–4.72)

Median and interquartile range of pupillometry assessments across use groups. Post 1 and Post 2 pupillometry assessments occurred approximately 40 min and 100 min, respectively, after first cannabis inhalation for the cannabis use groups. For the non-use group, the Post 1 and Post 2 pupillometry measurements occurred at equivalent times after a 15 min rest interval in which they did not use cannabis.

Table 4. Performance metrics and thresholds of pupillometer measures at Post 1 and Post 2, and least absolute shrinkage and selection operator (LASSO) models.

	Area under receiver operator characteristic curve (0–1.0)	Sensitivity	Specificity	Accuracy	Threshold
Post 1 assessment					
Maximum pupil diameter (mm)	0.53	0.83	0.35	0.71	<6.65
Minimum pupil diameter (mm)	0.60	0.67	0.55	0.64	>3.35
Percent change in pupil size (%)	0.73	0.76	0.61	0.72	>-43.11
Pupil latency (sec)	0.52	0.42	0.68	0.48	<0.09
Average pupil constriction velocity (mm/sec)	0.63	0.58	0.68	0.60	>-1.97
Maximum pupil constriction velocity (mm/sec)	0.65	0.59	0.71	0.62	>-4.10
Recovery dilation velocity (mm/sec)	0.60	0.48	0.74	0.55	<0.48
Recovery dilation area under the curve (mm x sec)	0.69	0.75	0.61	0.71	<4.61
Post 2 assessment					
Maximum pupil diameter (mm)	0.56	0.68	0.48	0.63	>5.86
Minimum pupil diameter (mm)	0.70	0.57	0.74	0.61	>3.65
Percent change in pupil size (%)	0.75	0.57	0.87	0.64	>-39.61
Pupil latency (sec)	0.58	0.47	0.68	0.52	>0.11
Average pupil constriction velocity (mm/sec)	0.61	0.33	0.87	0.46	>-1.82
Maximum pupil constriction velocity (mm/sec)	0.57	0.14	1.00	0.35	>-3.14
Recovery dilation velocity (mm/sec)	0.50	0.58	0.48	0.56	<0.55
Recovery dilation area under the curve (mm x sec)	0.66	0.48	0.81	0.56	<3.72
LASSO models					
LASSO: Post 1	0.79	0.73	0.74	0.73	--
LASSO: Post 2	0.75	0.79	0.65	0.75	--

flower group and 6.21 mm for the daily concentrate group.

Next, we considered the sensitivity, specificity, accuracy, and AUC for each pupillary variable, distinguishing the dichotomous outcome: recent cannabis inhalation versus no recent cannabis use, at each of the two post-use assessment points using data from the cannabis use groups and the non-using control participants (Table 4). The participants who used cannabis were combined, consistent with a forensic context in which a detailed history of cannabis use frequency would not be known. The pupillary

Table 5. Coefficient values for the least absolute shrinkage and selection operator (LASSO) models at each post-consumption assessment.

Pupil variable	Post 1 LASSO β	Post 2 LASSO β
(Intercept)	12.153	3.83
Maximum pupil diameter (mm)	−0.823	–
Minimum pupil diameter (mm)	−0.001	0.171
Percent change in pupil size (%)	0.266	0.082
Pupil latency (sec)	−3.338	–
Average pupil constriction velocity (mm/sec)	−3.557	–
Maximum pupil constriction velocity (mm/sec)	–	–
Recovery dilation velocity (mm/sec)	−1.919	–
Recovery dilation area under the curve (mm x sec)	−0.234	–

measures with the highest AUC value at the Post 1 assessment was percent change in pupil size (0.73), which had an accuracy of 72%, followed by recovery dilation AUC (AUC of 0.69, with 71% accuracy). At the Post 2 assessment, the variable with the highest AUC value was similarly the per percent change in pupil size (AUC of 0.75, and 64% accuracy), followed by the minimum pupil diameter (AUC of 0.70, with 61% accuracy). Table 4 also presents the threshold value of the variable associated with the AUC, sensitivity, specificity, and accuracy values. For example, at the Post 1 assessment, the threshold value of greater than −43.11% decrease in pupil size was associated with the AUC value of 0.73, meaning that individuals with larger values (less negative, and therefore less constriction) would be categorized as having recently used cannabis. As another example, at the Post 1 assessment, a threshold for minimum pupil size of greater than 3.35 mm indicates that individuals with a larger minimum pupil size would be categorized as having recently used cannabis.

Results of least absolute shrinkage and selection operator (LASSO) models

The logistic regression LASSO models shown in Table 5 selected a combination of pupil variables that define the highest discrimination ability between recent cannabis use and no use. In the first post-consumption assessment model, the LASSO selected all the variables except maximum pupil constriction velocity for inclusion in the model (Table 5). This model yielded an area under the receiver operator characteristic (ROC) curve of 0.79 (Table 4), which was higher than all the single variable models shown in Table 4. The Post 2 LASSO model (Post 2; Table 5) only selected minimum pupil diameter and percent change in pupil size, yielding an area under the ROC curve of 0.75 (Table 4). That value was the same as the single-variable model for percent change in pupil size at the assessment time shown in Table 4.

Exploratory analysis

Lastly, we considered the associations between blood delta-9-tetrahydrocannabinol concentrations and the analytic variables included in the LASSO models. We plotted these associations for Post 1 and Post 2, using log blood delta-9-tetrahydrocannabinol concentrations. For some variables, we found some small but significant positive associations (see Supplemental Figure 1).

Discussion

Our study investigated pupil size and pupillary dynamics via a research-grade pupillometer following observed and naturalistic use of various forms of inhaled cannabis. Prior research, with subjective observers, has suggested that cannabis may affect the resting pupil size. This may be operationalized variably, however, in this study, we measured the pupil diameter in darkness, after a rest period, which we refer to as maximum pupil size. We did not observe baseline differences which suggests that chronic use alone, in the absence of recent acute dosing, does not significantly impact this measure. Our finding for maximum pupil diameter following cannabis inhalation found that the cannabis use groups did not exhibit a substantial and consistent difference in static pupil diameter relative to controls, a finding also reported in other studies [17,19,20]. Inter-group differences that were observed were on average less than 0.2 mm and are unlikely to be discernible on unaided physical examination.

When considering the AUC of the individual dynamic pupillary variables, we found several measures that exceeded 0.65 on at least one of the two time points, such as the percent change from maximum to minimum pupil size. We utilized the LASSO model approach to leverage the full set of variables derived from the pupillometer with attention to a parsimonious model. This approach selected a model including nearly all the variables, at the first post-use time point, to achieve an AUC of 0.79. However, this might not be interpreted as substantially better than the single variable of percent change in pupil size, which, on its own, had an AUC of 0.73. Recovery dilation, assessed in this study

as the AUC of the pupil diameter trajectory from the inception of brief pupil illumination to the end of the test, was greater in the non-user group compared to the cannabis use group at both post-smoking assessments (indicating greater pupillary dynamics in the non-use group). A similar finding was observed in the investigation by Godbole et al. [23], which calculated rebound dilation by percent change in pupil size over time rather than in units of millimeters by time.

Other similar studies have focused on initial pupil size in a variety of lighting conditions and found mixed findings. Our study contributes to this literature by finding that the maximum pupil diameter (measured in darkness) was not the most informative pupillary measure. Rather, our results suggest that the percent change from maximum to minimum pupil size, after a brief bright light stimulus, appears to be more informative. These findings are most similar to those of Fant et al. [20], who reported that the pupil response to light was diminished after cannabis use, although that study was limited to 10 participants.

The pupillometer we used measured pupil size and did not measure other aspects of ocular function, such as measures of eye saccade. A systematic review [6] of psychotropic drugs and ocular effects identified some limited evidence that acute cannabis use can affect saccade reaction time and accuracy. Other research has identified that cannabis can affect visual function, such as contrast sensitivity. These parameters may also be of interest in future research and attempts to operationalize a test of acute cannabis.

Limitations

There are several limitations to this study, and limitations to the ability to apply these findings to real-world settings, such as use by law enforcement or in occupational settings. First, pupil size and response to light can be influenced by various individual characteristics, other drugs, and context factors. Our study had a limited focus on the acute effects of cannabis, but in real-world applications, there may be co-use of alcohol and other drugs that could alter the pattern of findings. Although we have relative balance by group by gender and age, this was an observational study and did not randomly assign participants to cannabis use groups. As a strength, we used naturalistic cannabis use, representing products available in a legal adult-use marketplace and a highly standardized protocol. However, participants used cannabis knowing they were part of this study and may have either chosen to use less than they otherwise would, knowing there would be assessments, or they may not have made the decision to drive after use in a real-world setting. Thus, there is the potential to both overestimate and underestimate the implications for real-world impaired driving behavior.

Additionally, the testing setting may not generalize to that more typical of a safety incident, such as a workplace injury or car crash, particularly considering the time points of 40 min and 100 min after use considered in this study. There is limited ability to know the window of time since cannabis use that a driver may be assessed for impairment after cannabis use. For example, one study found that the time from law enforcement dispatch to a blood draw, in cases of suspected driving under the influence vehicular homicide, was over 2 h [25]. If this same amount of time elapsed before an ocular assessment, then the observed effects could be even smaller than what we observed.

Our analysis approach sought to identify the optimal balance of sensitivity and specificity by considering the AUC and accuracy of the ocular parameters. However, depending on the testing context, there may be a higher priority for specificity (or sensitivity), and future analysis may consider these trade-offs differently. As there is a greater risk of consequences, such as legal consequences for a driver suspected of driving under the influence of drugs, there needs to be a corresponding increase in certainty of impairment status. For example, alcohol breathalyzers used by law enforcement have high sensitivity and specificity and can be used as evidence in court [26]. Our results do not suggest that ocular measures presented in this study are close to this level of prediction.

Conclusion

Pupillary dynamics, when measured with an objective test, may contribute to providing an indication of recent cannabis inhalation. These measures, such as constriction in response to light and recovery dilation, provide additional information beyond static pupil diameter. Further research would be necessary to understand the time interval following acute cannabis use during which pupillary measures have optimal predictive utility.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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