Drug and Alcohol Dependence

A pilot study of pupil response to light as a digital biomarker of recent cannabis use

--Manuscript Draft--

Manuscript Number:	
Article Type:	Full Length Article
Section/Category:	Epidemiology
Keywords:	pupillary light reflex; pupillometry; cannabis; functional data analysis; substance use detection
Corresponding Author:	Suneeta Godbole, M.S. Colorado School of Public Health Department of Biostatistics & Informatics UNITED STATES
First Author:	Suneeta Godbole, M.S.
Order of Authors:	Suneeta Godbole, M.S.
	Andrew Leroux, PhD
	Ashley Brooks-Russell, PhD
	Prem S. Subramanian, MD, PhD
	Michael J. Kosnett, MD, PhD
	Julia Wrobel, PhD
Abstract:	Introduction: Given the roadside safety and occupational injury prevention implications associated with cannabis impairment, there is a need for objective and validated measures of recent cannabis use. Pupillary light response may offer an approach for detection. Method: 84 participants (mean age: 32, 42% female) with daily, occasional, and no-use cannabis use histories participated in pupillary light response tests before and after smoking cannabis ad libitum or relaxing for 15 minutes (no use). The impact of recent cannabis consumption on trajectories of the pupillary light response was modeled using functional data analysis tools. Logistic regression models for predicting recent cannabis use were compared, and average pupil trajectories across cannabis use groups and times since light test administration were estimated. Results: Models revealed small, significant differences in pupil response to light after cannabis use comparing the occasional use group to the no use control group, and similar statistically significant differences in pupil response patterns comparing the daily use group to the no use comparison group. Trajectories of pupillary light response estimated using functional data analysis found that acute cannabis smoking was associated with less initial and sustained pupil constriction compared to no cannabis smoking. Discussion: These analyses show the promise of pairing pupillary light response and functional data analysis methods to assess recent cannabis use.
Suggested Reviewers:	Jihui Lee, Ph.D. Assistant Professor, University of Pittsburgh jihui.lee@pitt.edu Dr. Lee has expertise in the statistical field of functional data analysis and experience with digital biomarker.
	Patricia Di Ciano, PhD Assistant Professor, University of Toronto patricia.diciano@camh.ca Dr. Di Ciano has expertise in the effects of cannabis use.
	Bruna Brands, PhD Professor, University of Toronto bruna.brands@camh.ca Dr. Brands is an expert in the effects of cannabis use on road safety.
	Doug Beirness, PhD

	Senior Research Associate, Canadian Centre on Substance Use and Addiction dbeirness@ccsa.ca Dr. Beirness has expertise in the effects of impaired driving.
	Jeff Brubacher, MD Associate Professor, The University of British Columbia jeff.brubacher@ubc.ca Dr. Brubacher's research interest focuses broadly on injury prevention with a special interest in drug impaired driving, substance abuse and injury.

Suneeta V. Godbole
Department of Biostatistics & Informatics
Colorado School of Public Health
Fitzsimons Building, 4th Floor
13001 E. 17th Place
Mail Stop B119
Aurora, CO 80045

October 5, 2023

Dear Dr. Shoptaw,

We would like to submit for your review an original research article entitled, "A pilot study of pupil response to light as a digital biomarker of recent cannabis use" for consideration in *Drug and Alcohol Dependence*.

We confirm that this work is original and has not been published elsewhere nor is it currently under consideration for publication elsewhere.

Our paper provides new promising evidence for an objective digital marker of recent cannabis use that could be used to detect recent drug use for roadside safety and injury prevention. In this paper, we identify differences in trajectories of pupil response to light for participants who recently consumed cannabis compared to participants who did not. We are able show differences in trajectories based solely on the pupillary light response after smoking cannabis regardless of cannabis use history (i.e., not limited by drug tolerance). Results from this pilot study show promise that pupillary light response may be an objective, digital, and validated measure of recent cannabis use, making it an asset in the prevention of cannabis-involved roadside accidents and workplace injuries.

We have no conflicts of interest to disclose.

Please address all correspondence concerning this manuscript to suneeta.godbole@cuanschutz.edu.

Thank you for your consideration.

Sincerely,

Suneeta V. Godbole

Highlights:

- Results reveal differences in pupillary light responses between recent cannabis users and non-users.
- Daily and occasional users showed no difference in pupillary light response due to acute cannabis use, suggesting its use as a potential biomarker of recent use, invariant to cannabis tolerance.
- Models using functional data methods have promise for improving prediction over models using single value summaries.

A pilot study of pupil response to light as a digital biomarker of recent cannabis use

Title: A pilot study of pupil response to light as a digital biomarker of recent cannabis use

Authors: Suneeta Godbole[a]*, Andrew Leroux[a], Ashley Brooks-Russell[b], Prem S. Subramanian[c,d], Michael J. Kosnett[e,f], and Julia Wrobel[g]

Affiliations:

[a]Department of Biostatistics and Informatics, Colorado School of Public Health, University of Colorado Anschutz Medical Campus, Aurora, Colorado (suneeta.godbole@cuanschutz.edu; andrew.leroux@cuanschutz.edu)

[b]Injury and Violence Prevention Center, Colorado School of Public Health, University of Colorado Anschutz Medical Campus, Aurora, Colorado (ashley.brooks-russell@cuanschutz.edu)

[c]Departments of Ophthalmology, Neurology, and Neurosurgery, Sue Anschutz-Rodgers Eye Center, CU School of Medicine, University of Colorado Anschutz Medical Campus, Aurora, Colorado (prem.subramanian@cuanschutz.edu)

[d]Department of Surgery, Uniformed Services University of the Health Sciences, Bethesda, Maryland [5]Department of Medicine, CU School of Medicine, University of Colorado Anschutz Medical Campus, Aurora, Colorado

[e]Department of Medicine, CU School of Medicine, University of Colorado Anschutz Medical Campus, Aurora, Colorado (michael.kosnett@cuanschutz.edu)

[f]Department of Environmental and Occupational Health, Colorado School of Public Health, University of Colorado Anschutz Medical Campus, Aurora, Colorado

[g]Departement of Biostatistics and Bioinformatics, Emory University, Atlanta, Georgia (julia.wrobel@emory.edu)

*__Corresponding Author Email__: suneeta.godbole@cuanschutz.edu

Address: Department of Biostatistics & Informatics
Colorado School of Public Health
Fitzsimons Building, 4th Floor
13001 E. 17th Place
Mail Stop B119
Aurora, CO 80045

FUNDING: This work was supported by the Colorado Department of Public Health and Environment and the National Institutes of Health (R01DA049800).

A pilot study of pupil response to light as a digital biomarker of recent cannabis use

Abstract:

Introduction: Given the roadside safety and occupational injury prevention implications associated with cannabis impairment, there is a need for objective and validated measures of recent cannabis use. Pupillary light response may offer an approach for detection.

Method: 84 participants (mean age: 32, 42% female) with daily, occasional, and no-use cannabis use histories participated in pupillary light response tests before and after smoking cannabis *ad libitum* or relaxing for 15 minutes (no use). The impact of recent cannabis consumption on trajectories of the pupillary light response was modeled using functional data analysis tools. Logistic regression models for predicting recent cannabis use were compared, and average pupil trajectories across cannabis use groups and times since light test administration were estimated.

Results: Models revealed small, significant differences in pupil response to light after cannabis use comparing the occasional use group to the no use control group, and similar statistically significant differences in pupil response patterns comparing the daily use group to the no use comparison group. Trajectories of pupillary light response estimated using functional data analysis found that acute cannabis smoking was associated with less initial and sustained pupil constriction compared to no cannabis smoking.

Discussion: These analyses show the promise of pairing pupillary light response and functional data analysis methods to assess recent cannabis use.

KEYWORDS: pupillary light reflex, pupillometry, cannabis, functional data analysis, substance use detection

A pilot study of pupil response to light as a digital biomarker of recent cannabis use

1. INTRODUCTION:

According to the National Survey on Drug Use and Health, the rates of cannabis consumption have increased in adults over 26, and adults aged 18-25, from 4.0% to 7.9% and from 17.3% to 22.1% from 2002 to 2017, respectively (*Key Substance Use and Mental Health Indicators in the United States: Results from the 2017 National Survey on Drug Use and Health*, 2017). Along with increases in consumption, there have been increases in cannabis-involved motor vehicle fatalities from 9.0% in 2000 to 21.5% in 2018 (Lira et al., 2021; Myran et al., 2023). Although there is mixed evidence with regard to risk of occupational injury, with temporality of exposure being a major limitation of the extant literature (Biasutti et al., 2020; Zhang et al., 2020), cannabis consumption at or before work is of concern to employers, especially for employees involved in safety sensitive tasks. An objective, easy to obtain biomarker of recent cannabis use may be of value in field assessments, such as in the context of investigation of motor vehicle crashes and occupational incidents.

Current methods used to enforce existing regulations on drug impaired driving have multiple limitations for assessing recent cannabis use and impairment. The Standardized Field Sobriety Test is a general test for alcohol and drug impairment, comprised of the horizontal gaze nystagmus, walk and turn and one-leg stands (*Drug Evaluation and Classification (Preliminary School): Participant Manual*, 2015). While shown to be an accurate and reliable assessment for alcohol impairment, it has limited ability to identify recent cannabis use (Downey et al., 2012). In addition, many assessment tests have shown a reduction in effectiveness when administered to frequent cannabis users due to drug tolerance, leading to potential false negative results for frequent users (Arkell et al., 2021; Wurz and DeGregorio, 2022). Many states and countries reference drug levels in the blood as a threshold for impairment, much like the .08% blood

alcohol concentration level used as a *per se* definition of alcohol impairment in the U.S. Specific to cannabis, the parallel would be the blood level of delta-9-THC; however predictive models have better performance in participants abstaining for several days compared to those who exhibit more frequent or daily use (Burt et al., 2021). This is in part due to the fact that frequent users can maintain elevated levels of blood THC for days or weeks after consumption; as such, frequent cannabis users may have a blood test positive for THC even if they have not recently smoked cannabis (Burt et al., 2021). Given the limitations of blood THC levels and existing roadside assessments, there is a need for the development of objective markers of recent cannabis use and impairment from cannabis use.

Drug Recognition Experts, specially trained law enforcement officers, have included pupillary and ocular signs as indicators of the pharmacodynamic effects of drugs and alcohol (*Drug Evaluation and Classification (Preliminary School): Participant Manual*, 2015; Richman et al., 2004). They may examine pupil size under illumination ranging from near total darkness to bright light and assess the pupillary light reflex, which consists of constriction in response to visible light. This is similar to the pupillary light response test that is performed clinically to assess central nervous system function and acute drug effects. This test is administered by shining a light in the eye of the participant and measuring pupil size over the course of several seconds after the light is turned off. Studies assessing pupil size in cannabis users have yielded inconsistent results (Brown et al., 1977; Campobasso et al., 2020; Fant, 1998; Merzouki et al., 2008; Ortiz-Peregrina et al., 2020; Shahidi Zandi et al., 2021; Stark et al., 2003). Studies examining pupillary light response using device recorded, light-induced, pupil constriction have shown reduction in pupil diameter after cannabis use (Campobasso et al., 2020; Fant, 1998). Moreover, detailed assessment of the entire pupillary light response trajectory following acute

cannabis consumption is lacking. If the pattern of pupillary response to light were found to be indicative of recent cannabis use, or impairment from cannabis use, especially in the context of chronic frequent use, it could contribute to the assessment of impaired driving or have utility in investigations of cannabis use in the workplace and other safety sensitive settings.

Figure 1 shows a typical pupillary response to light during the light reflex test, which we refer to as a *pupillary light response trajectory* throughout the paper. After the light is shined the pupil begins to constrict in size until it reaches a minimum, called the *point of minimal constriction*, then it begins to increase in size back towards its original diameter. The area under the curve from the point of minimal constriction to the end of the light response test is known as the *rebound dilation*.

Recently, Steinhart et al (Steinhart et al., 2023) found that acute cannabis smoking was significantly associated with diminished pupillary constriction during a light response test conducted using infrared videography. Both occasional and daily cannabis users displayed this response, to a similar extent, compared to non-using control subjects. However, the findings of Steinhart et al, utilized single number summaries, such as point of minimal constriction, extracted from the full pupillary response trajectories. Ignoring these trajectories results in a loss of information that could potentially be utilized to better discriminate between recent cannabis use and no cannabis use, regardless of cannabis use history (daily versus occasional). Additionally, significant differences in the extent and pattern of pupillary constriction were only found after adjusting for pre-smoking values, which undermines the utility in field applications where baseline measurements may be unavailable.

The primary goal of this paper is to investigate the full pupillary light response trajectories collected in the study conducted by Steinhart et al (Steinhart et al., 2023) as

predictors of recent cannabis use, irrespective of pre-smoking information. Our analysis uses tools from a field of statistics known as functional data analysis (FDA). The main conceptual underpinning of FDA is to model the whole pupillary light response trajectory as a unit of observation, leveraging information contained in the temporal structure of the data and estimating time-specific effects. This approach utilizes maximal information which is lost when only considering single number predictors such as point of minimal constriction and rebound dilation (Goldsmith et al., 2011; Ramsay and Silverman, 2005). In this analysis, we will use FDA modeling techniques to accomplish the following objectives. We first use the full pupil light response trajectories to predict recent cannabis use as compared to no use. We next examine the impact of cannabis use history on the pupil response trajectories by comparing participants with a history of no recent cannabis use, occasional cannabis use, and daily cannabis use. Finally, we extract expected pupillary light response trajectories at 60, 65, and 70 minutes after cannabis use to explore how pupil response may change over time as a preliminary exploration of its utility.

2. METHODS:

2.1 Sample Information:

Data are from of a larger study examining effects of acute cannabis consumption among participants with occasional and daily cannabis use histories, to understand differences due to tolerance. Healthy adults were recruited using local ads, flyers, and email lists in the Denver area. Key eligibility criteria included ages 25 to 45 years old and willingness to use smoke cannabis and participate in study assessments. Key exclusion criteria include history of drug or alcohol dependence, currently pregnant, and body mass index above 35. Participants were recruited into one of three groups according to their history of cannabis use. Daily cannabis consumption was defined as smoking or vaping a cannabis flower product at least one time per

day, every day of the week for 30 days prior to enrollment. Occasional consumption was defined as smoking or vaping cannabis flower product on at least one day but no more than two days per week in the 30 days prior to enrollment. No cannabis consumption was defined as not having used cannabis in the month prior to enrollment.

Participants were instructed not to smoke cannabis for at least 8 hours, and not to use edible cannabis for at least 12 hours, before their data collection visit. This was verified by review of a history of the participant's cannabis use taken on the day of the visit. Each participant completed an alcohol breath test (Lifeloc FC10™) to screen for acute alcohol use, provided a urine sample to test for illicit drug use or use of prescription drugs not prescribed (30 mL Alere brand 13-panel iCup®).

The study utilized a within-subjects design comparing their pupillary light response before and after cannabis use. The cannabis use was observational in nature in that participants brought their own product, purchased from a state-license retail dispensary. Participants in the daily and occasional use groups were observed to smoke or vape cannabis flower during a 15-minute interval and were instructed to smoke *ad libitum* "the amount you commonly use for the effect you most commonly desire." Participants in the no use group were invited to relax for the equivalent amount of time. Written informed consent was obtained and the study was approved by the Colorado Multiple Institutional Review Board. More details on participant enrollment and screening criteria are previously published (Brooks-Russell et al., 2021).

Pupillary light response was collected from 101 participants. Upon inspection of the data, blinded to use group and/or time point of assessment, 18 were determined to have unusable data and were dropped from further analysis, resulting in a sample of 84 participants. Of the 84 participants used in this analysis, 29 were in the no-use group, and 30 and 25 participants in the

occasional and daily use groups, respectively. Participants ranged in age from 25.1 to 45.3 years with an average of 32 years (sd = 5.02); had an average BMI of 25.4 kg/m^2 (sd = 4.41); and were approximately 58% male (N = 49); see supplementary Table 1. THC levels were measured in whole blood collected 30 minutes after the inception of the 15-minute *ad-libitum* smoking interval. Time between cannabis smoking and the pupillary light response test varied from 53 – 84 minutes with a mean of 62.2 minutes (see Figure 4A). This time interval reflects the time used to complete other assessments such as a driving simulator test, as previously described (Brooks-Russell et al., 2021).

2.2 Pupil Response to Light Assessment:

FIGURE_1

Videos of pupil response during the light test were collected using SafetyScreen™ infrared video goggles developed by Ocular Data Systems, Inc (Pasadena, CA). Trajectories of pupil size during the light response test, like that shown in Figure 1, were extracted from the videos using the video segmentation pipeline described in Steinhart et al (Steinhart et al., 2023). These trajectories represent percent change in pupil size from the start of the light test, for the right eye, after cannabis consumption, in the occasional and daily use groups, and after a short rest period for the no use control group. Pupil light response trajectories were truncated to 400 frames, approximately 13.3 seconds after the start of the light test.

2.3 Functional Data Analysis

Functional data analysis (FDA) is a field of statistics that models functions (e.g. full trajectories/time series of pupillary light response) without extracting pre-defined specific features (Ramsay and Silverman, 2005). These functions may be either the outcome (the whole

trajectory is the outcome) or a predictor, or both. The methods are designed to handle complicated (e.g. highly non-linear) data and associations, while accounting for within person correlations over the function. In the current context, FDA methods allow for estimating and quantifying differences in how patterns of pupillary light response vary over time by cannabis use history. In our analysis, a single functional unit is the pupillary light response trajectory for a single subject. This functional unit is denoted $y_i(t)$ or $x_i(t)$ for participant *i*, depending on whether the trajectory is modelled as the outcome or predictor, respectively, with *t* specifying the time at which the measurement was assessed. For example, if a participant has the pupillary light response trajectory shown in Figure 1, with pupil change of -25.3% at 2 seconds after the start of the light test, then $y_i(t) = y_1(2) = -25.3$. Similarly, at 5 seconds after the start of the light test $y_1(5) = -14.9$.

Our analysis uses two distinct FDA methods to model differences in pupil response to light after cannabis use. The first method, functional logistic regression (also referred to as scalar-on-function regression in the FDA literature), is used to predict whether or not a subject recently used cannabis and treats the pupil response trajectory as a predictor variable. The second method, function-on-scalar regression, is used to model and visualize how patterns in the pupil response trajectories differ for participants with patterns of daily cannabis use, occasional cannabis use, and no use, and treats the pupillary light response trajectory as the outcome. These methods and their roles in this analysis are described in more detail below.

2.3.1 Predicting recent cannabis use via functional logistic regression

Here we use a functional logistic regression model to discriminate between those who recently smoked cannabis (designated "recent cannabis use," combining individuals with daily and occasional use patterns) and those who did not (designated "no use"). Functional logistic

regression (Goldsmith et al., 2011; Ramsay and Dalzell, 1991; Reiss et al., 2017) relates binary responses y_i (e.g. recent cannabis use vs. no use) to functional covariates $x_i(t)$ (the pupil response trajectory for the i^{th} participant). This model is analogous to logistic regression and is given by

$$logit\big(P(y_i = 1)\big) = \beta_0 + \int_t \beta_1(t)x_i(t). \quad (1)$$

The coefficient $\beta_1(t)$ can be thought of as a weight function, with larger absolute values indicating that pupillary light response (the functional covariate $x_i(t)$) is more strongly associated with the response (recent cannabis use) at a given time during the light test. As with traditional logistic regression, the coefficient $\beta_1(t)$ is interpreted as a log odds ratio of recent cannabis use associated with a 1% increase in pupil diameter; however, unlike traditional logistic regression, this log odds ratio is estimated at each time t during the pupil light response test. When exponentiated, $\beta_1(t)$ is interpreted as an odds ratio at each time t. The integral effectively takes a weighted average of the covariate effect over the test time. This model can be used to predict recent cannabis use using the full pupillary light response trajectory.

We compare the functional logistic regression model to a traditional logistic regression model that uses single value summaries of the trajectory data, including (a) minimal constriction, the magnitude of peak decrease in pupil diameter as a percentage of the pre-illumination diameter; (b) rebound dilation, the area under the curve of the relative pupil diameter after the point of minimal constriction (shaded in blue in Figure 1); and (c) the slope of the rebound from the point of minimal constriction to the end of the test as calculated in (Steinhart et al., 2023). For rebound dilation, a larger magnitude of area under the curve corresponds to less rebound dilation. We compare both models in their ability to predict recent cannabis use and expect better

prediction from the functional logistic regression model because it leverages information from the full pupillary light response trajectories. Area under the receiver operating characteristic curve (AUC) is used to compare the ability of each model to discriminate between recent cannabis use and no use, where values closer to 1 are interpreted as having a higher predictive accuracy. The statistical significance of differences between AUC curves was calculated with a Mann-Whitney U-statistic (DeLong et al., 1988).

2.3.2 Modeling patterns in pupil response trajectories across cannabis use groups

We use function-on-scalar regression (FoSR) to model average pupil response trajectories for participants with no cannabis use, patterns of occasional cannabis use, and patterns of daily cannabis use. FoSR is analogous to linear regression and relates functional responses $y_i(t)$ to scalar covariates x_i (e.g. age, cannabis use group, gender). In this analysis, we chose not to use demographic characteristics in our modelling because in field cannabis testing scenarios demographic information may not be known. The FoSR model is

$$y_i(t) = \beta_0(t) + \beta_1(t)I(use\ group = occasional) + \beta_2(t)I(use\ group = daily) + \varepsilon_i(t) \quad (2)$$

Indicators of cannabis use group are denoted by *I(use group = occasional)* and *I(use group = daily)*, which take values of 1 for subjects in the specified category and 0 otherwise. Coefficients $\beta_0(t)$, $\beta_1(t)$, and $\beta_2(t)$ are akin to regression coefficients in linear regression, with the added advantage that they are defined at each time t during the pupillary light response test. The intercept $\beta_0(t)$ is interpreted as the average trajectory of a participant in the no use control group. $\beta_1(t)$ is the average difference at a specific time *t* between the occasional use and no use groups, and $\beta_2(t)$ is the average difference between the daily use and no use groups. The error

term $\varepsilon_i(t)$, like in traditional linear regression, is normally distributed and independent across participants, but unlike traditional linear regression, the errors may be correlated over time *t*.

Modeling the effect of a time delay from cannabis use to testing pupillary light response

The time from cannabis use to the pupillary light response test ranged from 53 – 84 minutes (Figure 4A). We refer to this as the time delay (TD) and include it in a second FoSR model to explore how the shape of the pupil response trajectory changes over time as cannabis effects potentially become less pronounced. Cannabis use groups were combined to form one "recent use" group, which is compared with the no use group, and the time delay (TD) from cannabis use to testing was mean centered. This model is given by

$$y_i(t) = \beta_0(t) + \beta_1(t)I(recent\ use = 1) + \beta_2(t)I(recent\ use = 1) * TD + \varepsilon_i(t), \quad (3)$$

where $y_i(t)$, $\beta_0(t)$, and $\varepsilon_i(t)$ have the same interpretation as the previous FoSR model (Equation 2). $\beta_1(t)$ is interpreted as average difference in trajectories at a specific time *t* comparing recent cannabis use to no use with an average time delay from cannabis use to testing, and $\beta_2(t)$ is the average difference at a specific time *t* for an additional minute increase in time since smoking for the cannabis use group.

2.4 Analysis Software

All analyses were conducted using R version 4.0.2 (R Core Team, 2023). The R packages mgcv (Wood, 2017, 2011, 2004) and refund (Goldsmith, Jeff et al., n.d.)were used to implement functional data models. Estimation of the FoSR regression model follows the general algorithm presented by (Leroux et al., 2018). Code and data for reproducing our analysis is publicly available on GitHub.

3. RESULTS:

3.1 Sample

84 participants used in this analysis, there were 29 participants in the no-use group, and 30 and 25 participants in the occasional and daily use groups, respectively. Participants ranged in age from 25.1 to 45.3 years with an average of 32 years (sd = 5.02); had an average BMI of 25.4 kg/m^2 (sd = 4.41); and were approximately 58% male (N = 49); see Table 1. THC levels were measured in whole blood collected 30 minutes after the inception of the 15-minute *ad-libitum* smoking interval. Time between cannabis smoking and the pupillary light response test varied from 53 – 84 minutes with a mean of 62.2 minutes (see Figure 4A). This time interval was caused by normal variability in the time to complete other assessments in the study or to take breaks between assessments, as described in other results from the larger study (Brooks-Russell et al., 2021; Smith et al., 2023).

3.2 Predicting recent cannabis use

Figure 2A shows ROC curves that compare the ability of the functional and traditional logistic regression models to discriminate between recent cannabis use and no use. The functional logistic model, which uses the full pupillary light response trajectory, has a higher AUC value (AUC = 0.71) than the traditional logistic model based on single value summary features (AUC = 0.68). This indicates that the functional logistic regression model can better differentiate recent cannabis use from no use, although the difference is not statistically significant (p= 0.6) in this data set.

FIGURE_2

An added benefit of the functional logistic regression model is the ability to visualize the odds of cannabis use over the 10 seconds of the pupil light response test (Figure 2B). This plot shows two regions with statistically significant differences between recent cannabis use and no use. The first region between 2.03 and 3.73 seconds with a maximum difference at 2.97 seconds (OR: 2.66, 95% CI: [1.28, 5.50]) corresponds to the time period where the point of minimal constriction is typically observed and shows that individuals with less pupil constriction have higher odds of being in the cannabis use group. The second region between 5.7 and 7.3 seconds with a peak difference at 6.57 seconds (OR: 0.37, 95% CI: [0.17, 0.81]), occurs during the period of rebound dilation and shows that individuals with less pupil dilation (closer to the pupil diameter at the start of the test) have lower odds of being in the cannabis use group.

3.3 Visualizing patterns in pupil response trajectories across cannabis use groups

Figure 3 shows differences between the average trajectories of pupil light response in daily, occasional, and no-use groups estimated using the function-on-scalar regression (FoSR) model in Equation (2). The solid lines in Figure 3A represent estimated mean trajectories for those who did not use cannabis (purple line), for those in the occasional use group who recently smoked (light green line), and for those in the daily use group who recently smoked (dark green line). The dashed line in Figure 3A represents the estimated mean trajectory for all those who recently smoked (daily and occasional use groups combined). The no use group had a steeper decline in pupil size, more pupil constriction, and faster rebound dilation during the light test than the occasional or daily use groups. Estimated pupil trajectories for the occasional and daily use groups were similar, with marginally less constriction in the occasional use group.

Figure 3 panels B, C, and D show estimates and 95% confidence intervals for the average difference in pupil response for participants in the occasional vs no use groups, participants in

the daily vs. no use groups, and participants in the daily vs. occasional groups. Both Figure 3B and Figure 3C show regions of significant difference, indicating that there are significant differences in the average pupillary light response trajectory comparing recent cannabis use to no use, regardless of whether a participant had a history of occasional or daily cannabis consumption. Specifically, significant differences between the occasional and no-use groups are seen between 1.77 to 3.97 seconds with a peak difference at 2.87 seconds of 4.00% (95% CI: 1.32%, 6.68%), and between the daily and no-use groups between 2.1 to 2.73 seconds with a peak difference at 2.5 seconds of 2.88% (95% CI: 0.14%, 5.62%). Notably, no significant differences were found in the pupil response trajectories between the daily and occasional use groups, indicating that tolerance effects associated with daily use did not have a significant impact on pupillary light response in our data.

FIGURE_3

3.4 The effect of a time delay from cannabis use to testing pupil light response

Finally, we extracted expected pupil light response trajectories at 60, 65, and 70 minutes after cannabis use in an exploratory analysis of how pupil response changes farther out from the time of smoking. The number of minutes from cannabis smoking to administration of the pupillary light response test varied across study participants due to normal variability in the timing of study procedures, and we leverage this information to model how the pupil response trajectory is expected to change as time since cannabis smoking increases. Figure 4A shows the distribution of this time delay across subjects, which ranged from 53 to 84 minutes with a mean of 62.22 minutes (sd = 5.57 minutes). Figure 4B depicts the average trajectory for no cannabis use, and at 60, 65, and 70 minutes after cannabis use. It appears that after cannabis use, the point

of minimal constriction, and the extent of rebound dilation, approaches that of the no use group as time since cannabis consumption increases.

FIGURE_4

4. DISCUSSION:

There are several potential applications of an objective and non-invasive biomarker that could distinguish recent cannabis use with reasonable accuracy, such as forensic investigations in transportation crashes or workplace incidents. Our study explored the potential for trajectories of pupil size in response to light, as measured in a standardized way with infrared video goggles, to distinguish recent use from no recent us, among a sample of participants with a range of cannabis use histories. The current analysis suggests that pupillary light response, when paired with functional data analysis methods that leverage information from the full pupil response trajectory, has the potential to discriminate between participants who recently smoked cannabis and those with no history of recent use without needing pre-smoking data on pupillary light response.

To show the utility of using functional data analysis methods in predicting recent cannabis use, we compared the predictions from a functional logistic regression and traditional logistic regression model. While both models showed some predictive ability, the functional logistic regression model had a better predictive ability as indicated by higher AUC, although the difference between the models was not statistically significant in this pilot study. This better predictive ability may stem from the information that is retained when modelling full pupil trajectories versus the information loss that occurs when aggregating information into single summary values used in the traditional logistic regression framework. Additionally, the functional logistic regression was plotted to depict where and how the patterns of recent use and

no use groups differed significantly from each other. This plot showed two regions that were significantly different and corresponded to the point of minimal constriction and rebound dilation in typical pupillary light response trajectories. In the region of the point of minimal constriction, the model shows that less constriction is associated with higher odds of recently using cannabis, while in the region of rebound dilation, we see that less pupil dilation is associated with lower odds of recently using cannabis. This corresponds with previous evidence showing an effect of recent cannabis use on pupillary light response trajectories. However, the difference in predictive ability between the functional and traditional logistic regression were not statistically significant, which may be due to data quality and instrumentation difficulties as discussed in the limitations section.

Additionally, FDA methods allow interpretable visualization and statistical comparison of the average pupil responses across cannabis use groups. We found significant differences in pupil response, after cannabis smoking or an equivalent rest period, between the occasional and no use groups for time periods that correspond to the point of minimal constriction. This difference remained significant when comparing the daily use and no-use controls but was not significantly different when comparing the daily use and occasional use groups. These differences may be due to more dynamic pupil movements in non-users compared to cannabis users. Taken together, this provides promising evidence that the pupillary light response trajectory may be a measure of recent cannabis use that has utility in individuals with different cannabis use histories. We were also able to model and visualize how pupil response trajectories change as time since cannabis smoking increases. As expected, the pupil response trajectories for the cannabis smoking group appear to approximate the average trajectory of the no-use group as the time since smoking increases, especially in the region of the point of minimal constriction;

however, the slope of rebound dilation appears to remain distinct. The results were consistent with the hypotheses of differences in pupil light response by recent cannabis use, including frequent cannabis users, and a return to an average non-user trajectory with delayed test time.

There are several limitations to this analysis for which more sophisticated instrumentation and future data collection will be needed. Of primary concern were data quality issues that persisted after data processing, imputation and smoothing from the video segmentation pipeline. While most pupil light response trajectories reflected the characteristic pattern of the pupillary light response, there were a minority that were removed because there were no characteristic features of the light response. This led to a reduction in the sample size from a collection of 101 participants to usable data in 84. In addition, the non-standardized inter-subject geometry (pupil to camera distance) that characterized use of infrared videography instrumentation rendered it possible to assess change in pupillary diameter only as a percentage difference from baseline, and not in absolute size (mm). Baseline pupil diameter (in mm), which could not be measured in the present study may be an independent predictor of the pupillary light response (Larson and Behrends, 2015; McKay and Larson, 2021). Future research could examine the pupillary light response closer in time to smoking, and at a longer time interval following use to examine how the response changes over time. Despite these limitations, the results are promising for future research on pupillary changes associated with recent cannabis use.

This analysis is the first foray into pairing functional data analysis with pupillary light response trajectories to better understand the utility of these methods in detecting recent cannabis use. We are cautiously optimistic that these results suggest that, with further refinements, quantitative measurement and analysis of pupillary light response trajectory may aid the

objective assessment of recent cannabis use when only post cannabis use measurements can be obtained.

References:

Arkell, T.R., Spindle, T.R., Kevin, R.C., Vandrey, R., McGregor, I.S., 2021. The failings of *per se* limits to detect cannabis-induced driving impairment: Results from a simulated driving study. Traffic Injury Prevention 22, 102–107. https://doi.org/10.1080/15389588.2020.1851685

Biasutti, W.R., Leffers, K.S.H., Callaghan, R.C., 2020. Systematic Review of Cannabis Use and Risk of Occupational Injury. Substance Use & Misuse 55, 1733–1745. https://doi.org/10.1080/10826084.2020.1759643

Brooks-Russell, A., Brown, T., Friedman, K., Wrobel, J., Schwarz, J., Dooley, G., Ryall, K.A., Steinhart, B., Amioka, E., Milavetz, G., Sam Wang, G., Kosnett, M.J., 2021. Simulated driving performance among daily and occasional cannabis users. Accident Analysis & Prevention 160, 106326. https://doi.org/10.1016/j.aap.2021.106326

Brown, B., Adams, A.J., Haegerstrom-Portnoy, G., Jones, R.T., Flom, M.C., 1977. Pupil size after use of marijuana and alcohol. American Journal of Ophthalmology 83, 350–354. https://doi.org/10.1016/0002-9394(77)90732-2

Burt, T.S., Brown, T.L., Milavetz, G., McGehee, D.V., 2021. Mechanisms of cannabis impairment: Implications for modeling driving performance. Forensic Science International 328, 110902. https://doi.org/10.1016/j.forsciint.2021.110902

Campobasso, C.P., De Micco, F., Corbi, G., Keller, T., Hartung, B., Daldrup, T., Monticelli, F., 2020. Pupillary effects in habitual cannabis consumers quantified with pupillography. Forensic Science International 317, 110559. https://doi.org/10.1016/j.forsciint.2020.110559

DeLong, E.R., DeLong, D.M., Clarke-Pearson, D.L., 1988. Comparing the Areas under Two or More Correlated Receiver Operating Characteristic Curves: A Nonparametric Approach. Biometrics 44, 837. https://doi.org/10.2307/2531595

Downey, L.A., King, R., Papafotiou, K., Swann, P., Ogden, E., Boorman, M., Stough, C., 2012. Detecting impairment associated with cannabis with and without alcohol on the Standardized Field Sobriety Tests. Psychopharmacology 224, 581–589. https://doi.org/10.1007/s00213-012-2787-9

Drug Evaluation and Classification (Preliminary School): Participant Manual, 2015. . National Highway Traffic Safety Administration.

Fant, R., 1998. Acute and Residual Effects of Marijuana in Humans. Pharmacology Biochemistry and Behavior 60, 777–784. https://doi.org/10.1016/S0091-3057(97)00386-9

Goldsmith, J., Bobb, J., Crainiceanu, C.M., Caffo, B., Reich, D., 2011. Penalized Functional Regression. Journal of Computational and Graphical Statistics 20, 830–851. https://doi.org/10.1198/jcgs.2010.10007

Goldsmith, Jeff, Scheipl, F., Huang, L., Wrobel, J., Di, C., Gellar, J., Harezlak, J., McLean, M.W., Swihart, B., Xiao, L., Crainiceanu, C., Reiss, P.T., n.d. refund: Regression with Functional Data.

Key Substance Use and Mental Health Indicators in the United States: Results from the 2017 National Survey on Drug Use and Health (No. HHS Publication No. SMA 18-5068, NSDUH Series H-53), 2017. . Rockville, MD.

Larson, M.D., Behrends, M., 2015. Portable Infrared Pupillometry: A Review. Anesthesia & Analgesia 120, 1242–1253. https://doi.org/10.1213/ANE.0000000000000314

Leroux, A., Xiao, L., Crainiceanu, C., Checkley, W., 2018. Dynamic prediction in functional concurrent regression with an application to child growth. Statistics in Medicine 37, 1376–1388. https://doi.org/10.1002/sim.7582

Lira, M.C., Heeren, T.C., Buczek, M., Blanchette, J.G., Smart, R., Pacula, R.L., Naimi, T.S., 2021. Trends in Cannabis Involvement and Risk of Alcohol Involvement in Motor Vehicle Crash Fatalities in the United States, 2000–2018. Am J Public Health 111, 1976–1985. https://doi.org/10.2105/AJPH.2021.306466

McKay, R.E., Larson, M.D., 2021. Detection of opioid effect with pupillometry. Autonomic Neuroscience 235, 102869. https://doi.org/10.1016/j.autneu.2021.102869

Merzouki, A., Mesa, J.M., Louktibi, A., Kadiri, M., Urbano, G.V., 2008. Assessing changes in pupillary size in Rifian smokers of kif (Cannabis sativa L.) q. Journal of Forensic and Legal Medicine.

Myran, D.T., Gaudreault, A., Pugliese, M., Manuel, D.G., Tanuseputro, P., 2023. Cannabis-Involved Traffic Injury Emergency Department Visits After Cannabis Legalization and Commercialization. JAMA Netw Open 6, e2331551. https://doi.org/10.1001/jamanetworkopen.2023.31551

Ortiz-Peregrina, S., Ortiz, C., Castro-Torres, J.J., Jiménez, J.R., Anera, R.G., 2020. Effects of Smoking Cannabis on Visual Function and Driving Performance. A Driving-Simulator Based Study. IJERPH 17, 9033. https://doi.org/10.3390/ijerph17239033

R Core Team, 2023. R: A language and Environment for Statistical Computing.

Ramsay, J.O., Dalzell, C.J., 1991. Some Tools for Functional Data Analysis. Journal of the Royal Statistical Society: Series B (Methodological) 53, 539–561. https://doi.org/10.1111/j.2517-6161.1991.tb01844.x

Ramsay, J.O., Silverman, B.W., 2005. Functional Data Analysis, 2nd ed, Springer Series in Statistics. Springer, New York, New York, USA.

Reiss, P., T., Goldsmith, J., Shang, H.L., Ogden, R.T., 2017. Methods for scalar-on-function regression. International Statistical Review 85, 228–249. https://doi.org/doi:10.1111/insr.12163

Richman, J.E., Golden McAndrew, K., Decker, D., Mullaney, S.C., 2004. An evaluation of pupil size standards used by police officers for detecting drug impairment. Optometry - Journal of the American Optometric Association 75, 175–182. https://doi.org/10.1016/S1529-1839(04)70037-8

Shahidi Zandi, A., Comeau, F.J.E., Mann, R.E., Di Ciano, P., Arslan, E.P., Murphy, T., Le Foll, B., Wickens, C.M., 2021. Preliminary Eye-Tracking Data as a Nonintrusive Marker for Blood Δ-9-Tetrahydrocannabinol Concentration and Drugged Driving. Cannabis and Cannabinoid Research 6, 537–547. https://doi.org/10.1089/can.2020.0141

Smith, S.J., Wrobel, J., Brooks-Russell, A., Kosnett, M.J., Sammel, M.D., 2023. A Latent Variable Analysis of Psychomotor and Neurocognitive Performance After Acute Cannabis Smoking. Cannabis 6, 123–132. https://doi.org/10.26828/cannabis/2023/000156

Stark, M.M., Englehart, K., Sexton, B.F., Tunbridge, R., Jackson, P., 2003. Use of a pupillometer to assess change in pupillary size post-cannabis. Journal of Clinical Forensic Medicine 10, 9–11. https://doi.org/10.1016/S1353-1131(02)00162-1

Steinhart, B., Brooks-Russell, A., Kosnett, M., Subramanian, P., Wrobel, J., 2023. A Video Segmentation Pipeline for Assessing changes in Pupil Response to Light After Cannabis Consumption. Journal of Data Science.

Wood, S.N., 2017. Generalized Additive Models: An Introduction with R., 2nd ed. Chapman and Hall/CRC.

Wood, S.N., 2011. Fast Stable Restricted Maximum Likelihood and Marginal Likelihood Estimation of Semiparametric Generalized Linear Models. Journal of the Royal Statistical Society Series B: Statistical Methodology 73, 3–36. https://doi.org/10.1111/j.1467-9868.2010.00749.x

Wood, S.N., 2004. Stable and efficient multiple smoothing parameter estimation for generalized additive models. Journal of the American Statistical Association 99, 673–686.

Wurz, G.T., DeGregorio, M.W., 2022. Indeterminacy of cannabis impairment and Δ9-tetrahydrocannabinol (Δ9-THC) levels in blood and breath. Sci Rep 12, 8323. https://doi.org/10.1038/s41598-022-11481-5

Zhang, J.C., Carnide, N., Holness, L., Cram, P., 2020. Cannabis use and work-related injuries: a cross-sectional analysis. Occupational Medicine 70, 570–577. https://doi.org/10.1093/occmed/kqaa175

Figure Legends:

FIGURE 1: A typical pupillary response to light during the light reflex test, which we refer to as a *pupillary light response trajectory* throughout the paper. At the onset of illumination (time 0 on the x-axis) the pupil begins to constrict in size until the diameter reaches a minimum, called the *point of minimal constriction*, then it begins to increase in size back towards its original diameter. The area under zero on the y-axis from the point of minimal constriction to the end of the light response test is a measure of *rebound dilation*. The larger the magnitude of this area (i.e. larger shaded in Figure 1), the less rebound dilation that has occurred.

FIGURE 2: *Panel A*: Receiver Operator Characteristic curves (ROCs) for our two logistic regression (LogRegr) models. Higher accuracy in predicting recent cannabis use is indicated by a higher AUC and the ROC curve following the left and top edge of the graph. The blue line is an ROC curve for a traditional logistic regression model using single value summary features of pupil light response. The yellow line is an ROC curve for a functional logistic regression model using full trajectory of pupil light response. The functional logistic model better differentiates between recent cannabis use and no use. *Panel B*: Solid black line depicts the odds ratio (OR) of recent cannabis over the 10 seconds of the pupillary light response test. The dashed lines indicate the 95% confidence interval around the OR estimate. The red segments indicate regions where the confidence interval for the OR does not contain zero, demonstrating statistically significant differences between the recent cannabis use and no use.

FIGURE 3 A-D: Panel A shows average pupil light response trajectories plotted by cannabis use frequency. An additional dotted lined based on the average trajectory for all recent cannabis users, occasional and daily, was included to show differences between recent use and no use groups. Panels B - D show the difference in average trajectories between pairs of occasional,

daily and no-use of cannabis. Zero on the y-axis corresponds to no difference between the average trajectory of two groups, while the region indicated by the solid red line, where the confidence interval (both dashed lines) is above or below zero on the y-axis indicates statistically significant differences between trajectories. The figure demonstrates significant regions of difference between occasional and no-use groups and daily and no-use groups, while there is no significant difference between occasional and daily cannabis use groups.

FIGURE 4: *Panel A*: Histogram depicts the distribution of the time delay from cannabis use to the pupillary light response test, in minutes. The vertical dotted red line indicates the mean of the distribution at 62.2 minutes. Interquartile range is 59 – 66 minutes. *Panel B*: Differences in the average pupil light response as the time from cannabis smoking increases from 60 minutes to 70 minutes (lighter color). The purple line shows the average pupil response for the no use group. As time since cannabis consumption increases, the point of minimal constriction approaches that of the no use group while the slope of the rebound appears to remain distinct.

Figure_1

Click here to access/download;Figure;Figure1.jpeg

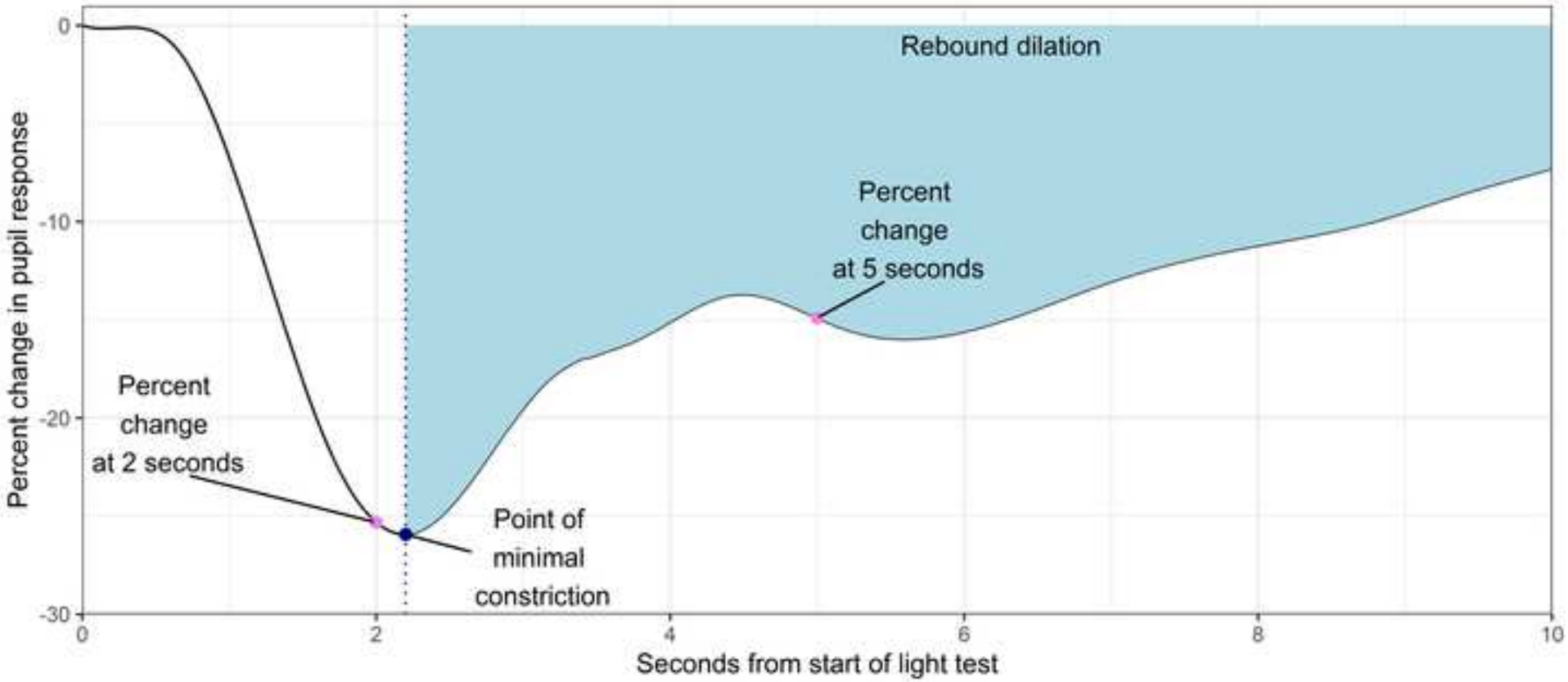


Figure_2

Click here to access/download;Figure;Figure2.jpeg

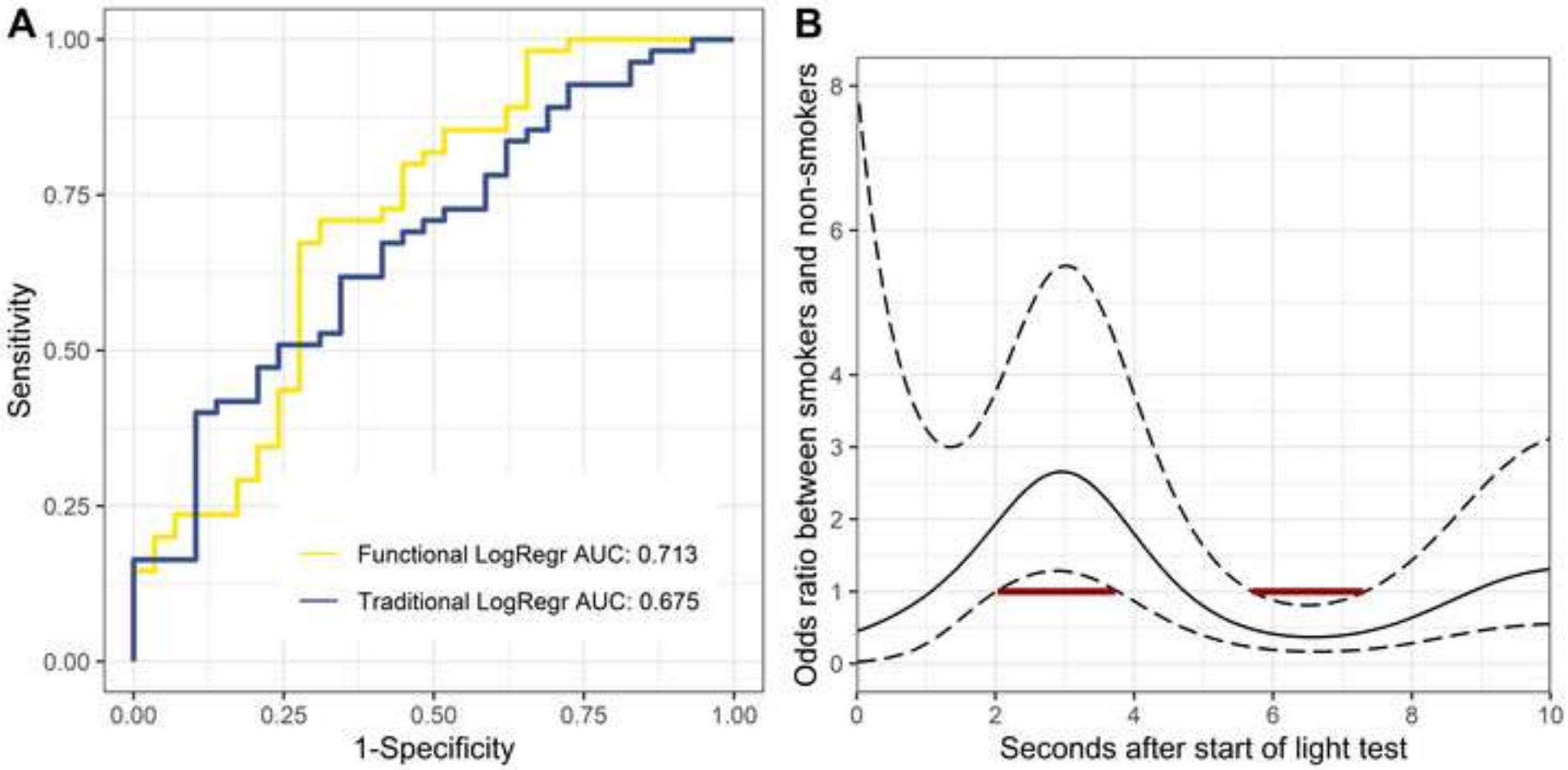


Figure_3

Click here to access/download;Figure;Figure3.jpeg

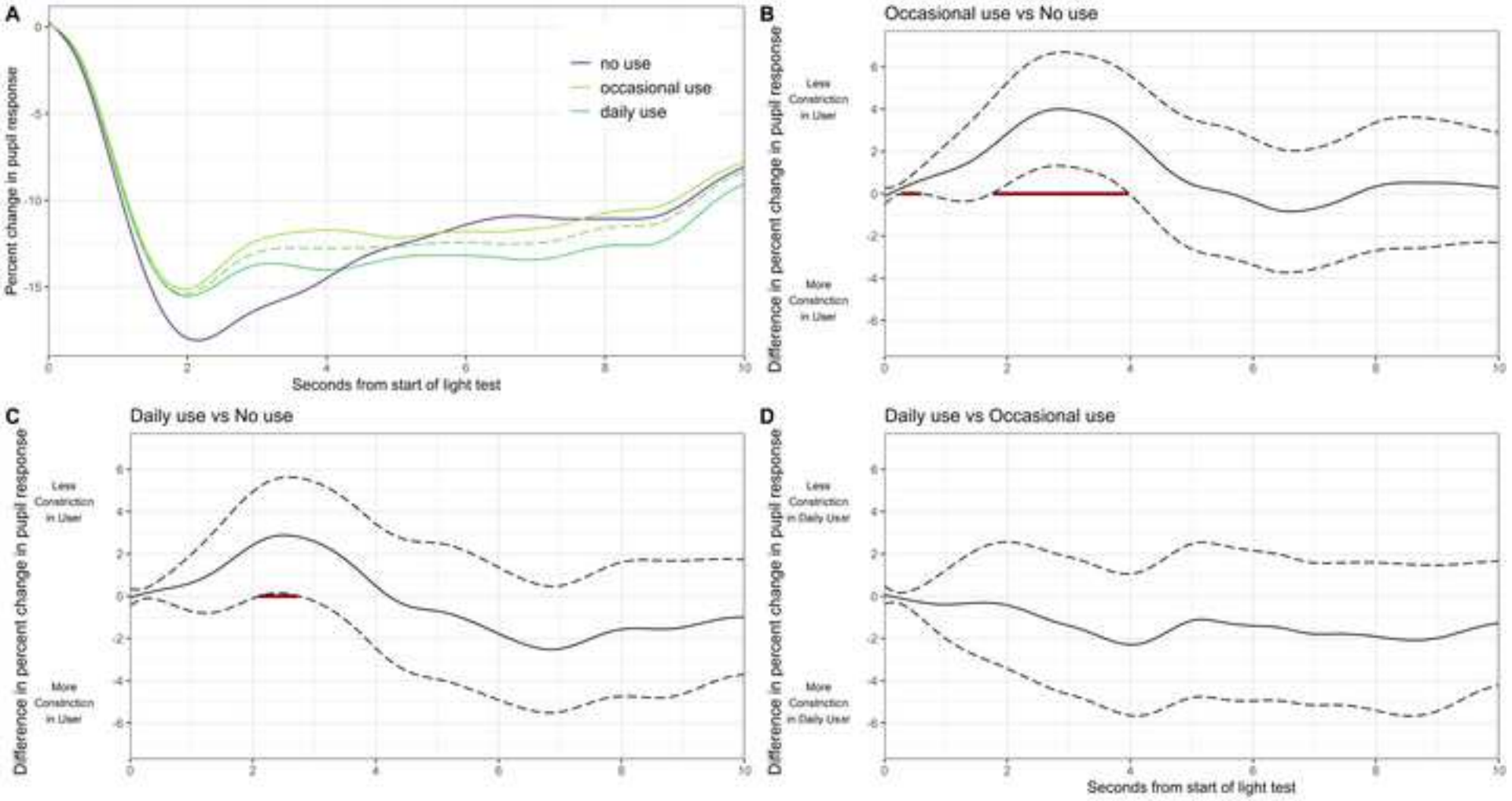
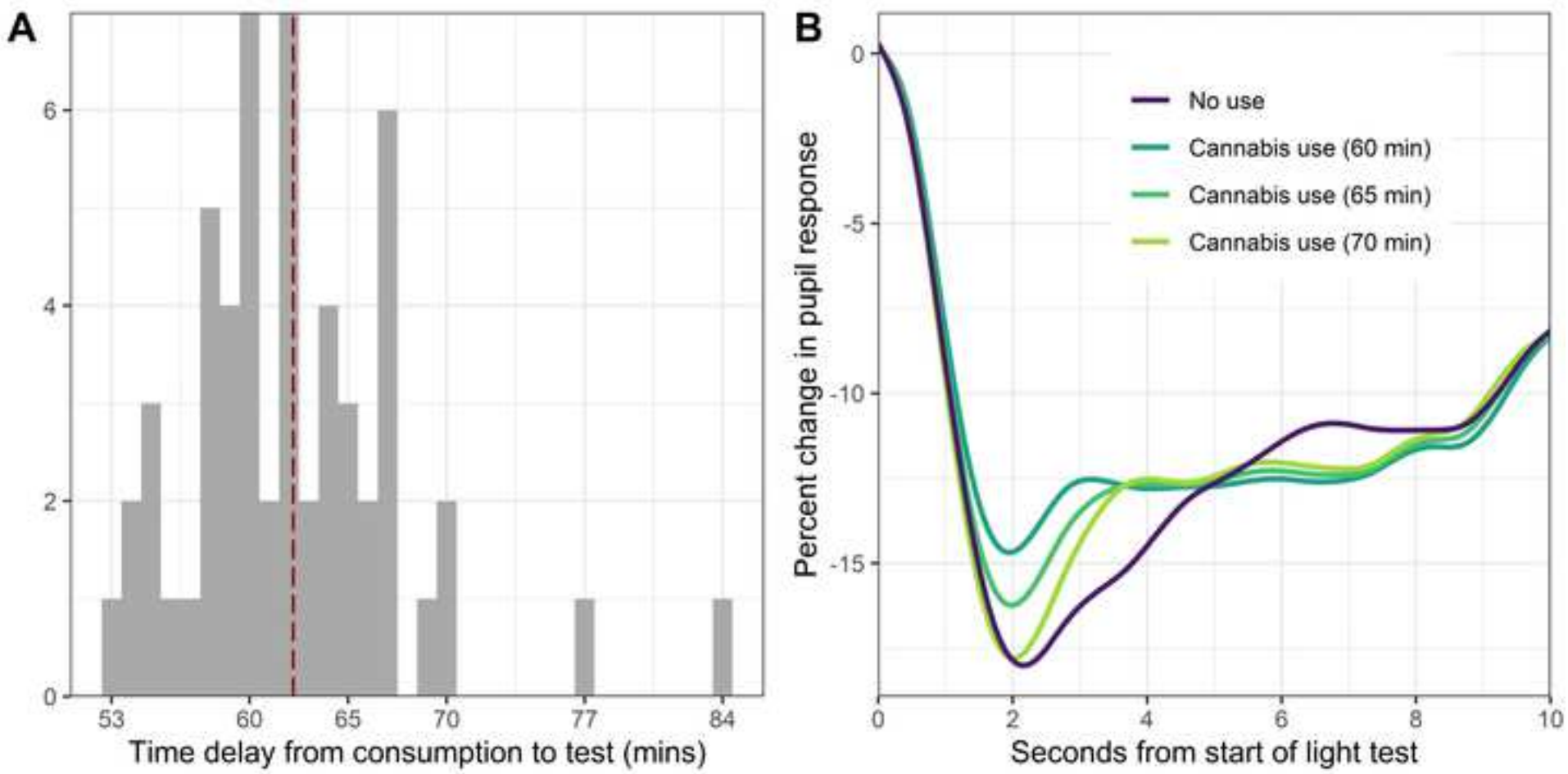


Figure_4



Characteristic	Cannabis Use Group				
	non-user (N = 29)[1]	**occasional** (N = 30)[1]	**daily** (N = 25)[1]	**Total** (N = 84)[1]	**p-value**[2]
Age (years)	32.29 (4.70)	31.15 (4.75)	32.75 (5.71)	32.02 (5.02)	0.5
Sex					0.2
Female	16 (55%)	10 (33%)	9 (36%)	35 (42%)	
Male	13 (45%)	20 (67%)	16 (64%)	49 (58%)	
Body Mass Index (kg/m^2)	24.94 (4.72)	24.49 (3.96)	27.08 (4.26)	25.42 (4.41)	0.066
THC, after cannabis smoking (ng/ml)	0.0 (0.0, 0.0)	5.73 (3.73, 9.47)	17.84 (8.20, 42.42)	3.96 (0.0, 13.60)	NA
Time Interval of pupillary measurements after initiation of cannabis smoking (mins)	NA	63.93 (6.26)	60.16 (3.78)	NA	

[1]Mean (SD); n (%); Median (IQR)

[2]Kruskal-Wallis rank sum test; Pearson's Chi-squared test

SUPPLEMENTARY TABLE 1: Participant Characteristics by Cannabis Use Group

Author Disclosures

Role of Funding Source:

Funders had no involvement in study design, data collection or analysis, writing the report, or the decision to submit the article for publication.

Contributors:

Ms. Godbole conducted data analysis, and manuscript writing. Drs. Brooks-Russell, Wrobel, Kosnett, and Subramanian participated in the study design and implementation, manuscript writing and editing. Drs. Wrobel and Leroux created the data analysis plan and supervised analysis. Dr. Leroux also contributed to the manuscript editing.

Conflict of Interest:

No conflict declared.

Contributors:

Ms. Godbole conducted data analysis, and manuscript writing. Drs. Brooks-Russell, Wrobel, Kosnett, and Subramanian participated in the study design and implementation, manuscript writing and editing. Drs. Wrobel and Leroux created the data analysis plan and supervised analysis. Dr. Leroux also contributed to the manuscript editing.

Declarations of interest: none.