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The effects of cannabis on sleep, circadian rhythms, and cognition in young adults

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The Effects of Cannabis on Sleep, Circadian Rhythms, and Cognition in Young Adults

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Abstract

Cannabis legalization is increasing steadily in the United States, coinciding with an increase in recreational use and THC potency. Chronic cannabis use is associated with altered sleep structure and mild cognitive deficits in learning and memory compared to non-users. Researchers have found evidence that chronic administration of THC disrupts circadian signaling on the molecular level (Lafaye et al., 2019), while others propose that chronic cannabis use may act as a signaling cue for rhythmicity (Whitehurst et al., 2015). This conceptual replication of Whitehurst's study investigates the effects of chronic cannabis use on sleep, circadian rhythm, and cognitive performance of young adults by comparing chronic users' sleep parameters, circadian rhythm patterns, and cognitive performance to non-cannabis using age-matched controls. Participants were given an actigraph device to monitor sleep and activity for two weeks, morning and evening sleep/substance use journals, sleep questionnaires, and the Automated Neuropsychological Assessment Metric (ANAM). Urinalyses were conducted to confirm group membership. No significant differences were identified between groups in sleep patterns, circadian rhythm, and cognitive performance. Within the chronic cannabis user group, frequency of reported use and years of chronic use displayed predictive properties for select cognitive domains and sleep parameters.

Introduction

The nature of cannabis use has changed over the last few decades. Daily cannabis use is the highest reported since 1983 while cannabis popularity is increasing both in use and acceptance of users. In contrast, alcohol and cigarette use is declining among young adults (National Institute on Drug Abuse, 2020). Nearly one in ten young adults aged 19-30 reported using cannabis daily in the latest publishing of Monitoring the Future, a survey conducted by the NIH (2020). Paralleling the increase in cannabis use is a decrease in perceived risk of regular cannabis use, continuing a steep decline that began in 2006. In 2006, about 56% of young adults perceived chronic cannabis use as a great risk to their health compared to 19-22% in the most recent edition (NIH, 2020). Moreover, state legalization of cannabis continues to spread across the United States, reflecting the perception of minimal risk from cannabis use. Currently, cannabis recreational or medical use is legal in 18 U.S. states (National Conference of State Legislatures), resulting in an increase in availability and correspondent rise in use. This increase in legalization coincides with an increase in social acceptance, especially in college students (Graupensperger, 2022).

As cannabis continues to become more widely available, more young adults and college students are using more regularly. The willingness to improve sleep is a large factor in this population. A 2018 survey found that up to 15% of college students use cannabis as a sleep aid (Goodhines et al.); however, many students who use alcohol and cannabis as sleep aids overestimate the benefits of each substance (Graupensperger, 2022). In fact, present research suggests that chronic cannabis use is associated with altered sleep structure and mild cognitive deficits in certain areas compared to non-users

(Lovell et al., 2020). Researchers have found evidence that chronic cannabis use may dysregulate circadian gene function (Lafaye et al., 2019), while other studies found it may facilitate circadian entrainment (Whitehurst et al., 2015). This study aims to explore whether the main findings of Whitehurst et al. can be repeated by investigating the efficacy of cannabis as a biological cue for circadian processes and examine the effects of cannabis on sleep and cognitive performance. The current study will observe chronic users as in Whitehurst et al. but with less experience and later onset of use to see if the effect can be repeated.

Circadian Rhythm

Circadian rhythms are internal diurnal rhythms coordinated by the mammalian brain and modulated by environmental cues (called "zeitgebers"), such as light, activity, and meals. The suprachiasmatic nucleus (SCN) of the anterior hypothalamus is recognized as the brain's internal clock structure, generating a consistent rhythm synchronized with these external cues via photoreceptive ganglion cells signaling through the retinohypothalamic tract, which is separate from visual networks (Rosenwasser & Turek, 2015). Reduction of light cues in the evening trigger the SCN to signal for melatonin release by the pineal gland. Melatonin is synthesized from serotonin in the pineal gland and a small part of the retina. It is involved in the circadian onset of sleep by initiating core body temperature decline and projecting to wake-promoting structures (Cagnacci et al., 1995).

The SCN is a small lobe on the lateral hypothalamus and has 20,000 neurons that individually generate high amplitude rhythms of gene expression and activity, mediated

by two interlocking transcription/translation feedback loops (Sollars & Pickard, 2015; Partch et al., 2014). Feedback loops are driven by the fluctuation of four core clock protein levels. The *Clock* and BMAL1 protein levels rise while PER and CRY levels fall over the 24-hour cycle (Partch et al., 2014). These molecular oscillations reflect SCN functional output changes to the circadian cycle (Sollars & Pickard, 2015). *Clock* genes are expressed not only in SCN neurons, but also the central nervous system and throughout peripheral tissues. Peripheral circadian regulation is facilitated by *Clock* genes in microglial cells (Lafaye et al., 2019), located in both tissues and organs.

Endocannabinoid System

The endocannabinoid system (ECS) is a complex neuromodulatory signaling system that regulates major neurotransmitter systems, maintaining homeostasis in response to environmental fluctuations (Antony et al., 2020). The ECS is unique from other neurotransmitter systems in that its endogenous ligands are synthesized and released on demand, rather than being synthesized ahead of time and stored in vesicles (Lu & Mackie, 2016). Endocannabinoids are released from postsynaptic cells, travelling in a retrograde direction to presynaptic CB1 receptors. They mediate incoming signals to the postsynaptic site, inhibiting the release of many excitatory and inhibitory neurotransmitters (Alger, 2013). The two ligands identified thus far for the ECS are anandamide and 2- arachidonoylglycerol (2-AG), which are lipid metabolites (Mechoulam et al., 1995; Sugiura et al., 1995). Anandamide is a low affinity agonist for the CB1 receptor, 2-AG is a high affinity agonist (Mechoulam et al., 1995). Different ligands similar in molecular structure to those in the ECS have been identified, but more

research must be done extricate their biological significance (Hanus & Mechoulam, 2010). Known endocannabinoid receptors CB1 and CB2 are prolifically distributed throughout the central nervous system (CNS) and periphery (Breivogel & Childers, 1998). Specifically, the CB1 receptor is found abundantly in the CNS, including the hypothalamus, mesolimbic pathway (reward center), cerebral cortex, amygdala, and parts of the brain stem. It is involved in regulating appetite, mood, reward, learning, memory, and sleep (Zou & Kumar, 2018), all of which are known to express rhythmicity. CB2 is more abundantly located in the periphery and mediates pain management, immune response, and tissue regeneration (Dedhia & Kole, 2021). In this study we are primarily concerned with CB1, because THC exerts its psychoactive effects by binding to this receptor (Babson et al., 2017).

The internal circadian structure (or "clock") of the brain is densely innervated by CB1 receptors. Endocannabinoids project from the internal clock to different regions of the brain responsible for carrying out rhythmic biological responses. This includes (but is not limited to) body temperature, peripheral metabolism, endocrine functioning, mood, cognition, and the sleep-wake cycle (Dedhia & Kole, 2021). Endocannabinoids follow a rhythm of activity, suggesting they are under the influence of circadian processes. For example, a study performed using rodent models showed increased levels of circulating anandamide in the nucleus accumbens, prefrontal cortex, striatum, and hippocampus during the dark phase of the 24-hour cycle; coupled with decreased levels of fatty acid amide hydrolase, which breaks down anandamide. The opposite showed true for 2-AG levels, peaking during the light phase in the same regions and lowering during the dark

phase (Valenti et al., 2004). The bidirectional relationship of circadian processing and the endocannabinoid system suggests that modification of one may duly influence the other.

Cannabis

The cannabis plant has at least 100 cannabinoids, 200 terpenes, flavonoids, and omega fatty acids, all of which contribute to different variations of the plant. Delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) are the most researched and recognized as the primary active compounds of cannabis flower. Cannabinol (CBN), cannabichromene (CBC), cannabigerolic acid (CBGA) have recently been isolated, yet their effects are not well researched yet (Dedhia & Kole, 2021).

The exact mechanisms of cannabinoid action are not well understood, but evidence supports the assertion that THC produces psychoactive effects by acting on CB1 receptors as a partial agonist. These effects include euphoria, distorted sensory perception, reduced motor activity and core body temperature, and distorted perception of time (Hall & Sowoji, 1998; Ashton, 2001). CBD mechanisms of action are not well understood due to inconsistent *in-vivo* and *in-vitro* studies. However, sufficient evidence suggests that CBD acts as a negative allosteric modulator of CB1 (Lapraire et al., 2015), reducing the efficacy of THC. This relationship supports the theory that CBD counteracts adverse effects of THC. While CBD has been shown to bind weakly to CB1 receptors, it binds with greater affinity to serotonin, dopamine, and opioid receptors (Mlost et al., 2020). CBD has no psychoactive effects, but rather evidence of anti-inflammatory, anti-psychotic, anxiolytic, and pain-reducing properties.

Terpenes are volatile oils found universally in flowering plants, responsible for unique aromas of each. Accordingly, different varieties of cannabis have distinctive terpenes. Some may increase the soporific (sleep-promoting) potential of cannabinoids, and some may enhance lipid solubility, facilitating infiltration to the CNS (Dedhia & Kole, 2021). Experts propose that whole plant extractions are more beneficial as compared to consuming isolated compounds of cannabis, because the components (cannabinoids, terpenes, flavonoids) work better in conjunction with each other. Consuming isolated cannabis compounds may result in adverse effects, as the compounds seem to ameliorate the others' potential to cause such outcomes. This concept is called the entourage effect, a theorized mechanism in which cannabis compounds act in synergy (Dedhia & Kole, 2021).

Circadian Rhythm and Cannabinoids

Circadian manipulation, meaning lengthening or shortening the circadian period of the 24-hour cycle, can be accomplished via exogenous administration of cannabinoids. For example, peripheral circadian regulation is facilitated by various *Clock* genes in microglial cells. Chronic use of THC leads to increased tolerance, on a molecular level this causes malfunctioned phosphorylation of, and consequently desensitized and downregulated CB receptors, including those on microglia. Likewise, chronic CBD administration was found to disrupt *Clock* gene expression in microglia, upregulating *Arntl* genes and downregulating *Clock* genes (Lafaye et al, 2019). Chronic circadian disruption is associated with a wide range of adverse health outcomes, including but not

limited to mood problems, cardiovascular disease, obesity, cognitive deficits, and more (Pacher & Kunos, 2013).

However, other evidence suggests that cannabis helps circadian entrainment. CB1 agonists alter the SCN's ability to entrain to environmental light cues by limiting GABA release in pre-synaptic axon terminals of the SCN, consequently driving SCN neural activity (Acuna-Goycolea et al., 2010). From this interaction, THC consumption disorients our perception of time, inhibiting light-induced phase shifts in rhythm. A phase shift indicates that one's rhythm has advanced, meaning bedtime and waketime occur earlier, or one's rhythm is delayed, indicating bedtime and waketime occur later in the 24-hour cycle. These findings support the theory that cannabis may serve as a zeitgeber (Whitehurst et al., 2015), facilitating circadian entrainment. Users in Whitehurst et al.'s study demonstrated more robust circadian entrainment than nonusers. Chronic user rhythms also fit better to the circadian cosine model, again showing more consistent rhythmicity.

Entrainment via Cannabinoids and Thermoregulation

Circadian oscillation of body temperature has been repeatedly linked to the sleep-wake cycle. Specifically, the undulation of body temperature may serve as an additional signaling mechanism for the sleep-wake rhythm (VanSomeren, 2009). Generation of sleep typically occurs on the steep decline of core body temperature (Krauchi & Wirz-Justice, 2001). Additionally, the sleep-promoting (soporific) effects of endogenous melatonin have been linked to hypothermic effects (Krauchi et al., 1997). Studies have also explored the soporific utility of exogenous melatonin supplementation, finding that

doses exceeding 1mg resulted in coinciding sleep-inducing and hypothermic effects in young adult volunteers (Cagnacci et al., 1992). In 2004, researchers in Australia recognized that other soporific drugs, namely benzodiazepines, possibly exert their effects via hypothermia (Gilbert et al). They administered 5mg of melatonin, 20 mg of a benzodiazepine (temazepam), or a placebo to healthy young adult subjects in a double-blind, counterbalanced design. Subjects underwent core body temperature monitoring and sleep latency testing via the Multiple Sleep Latency Test. Each treatment reduced core body temperature and sleep latency significantly as compared to the placebo condition. A meaningful linear relationship was also identified between maximal sleepiness and maximal rate of core temperature decline in both the melatonin condition ($r = .48$) and temazepam ($r = .44$).

Cannabinoids mediate hypothermic and/or anti-inflammatory mechanisms, exerting neuroprotective effects on the CNS (Pacher & Kunos, 2013). Cannabinoids generate hypothermia by altering projections from the hypothalamus, inducing CB1 receptor-mediated hypothermia (Pacher & Kunos, 2013), as elucidated by multiple animal and human studies (Pertwee, 1985; Pertwee, 1992; Sulcova et al, 1998; Smirnov & Kiyatkin, 2008). For example, researchers intraperitoneally injected 3 differing doses of THC (0.5, 2.0, 8.0 mg/kg) into rodents and observed the consequent locomotive and thermic effects 60-, 120-, and 180 minutes post-injection. Brain, muscle, and skin temperature and locomotion decreased significantly compared to control in the 2.0 and 8.0mg/kg conditions, with the 8.0 mg/kg showing the strongest difference at the 60-minute mark (Smirnov & Kiyatkin, 2008).

Hodges & Ashpole (2019) reviewed literature on aged individuals' circadian rhythms and ways in which cannabinoids could augment them. The authors note that circadian rhythm of peripheral tissues runs close to 24 hours but can be modified by thermoregulatory signals sent from the SCN. Pharmacological manipulation that acts on SCN-regulated body temperature could then work effectively by shifting peripheral rhythm. While the hypothermic effect of cannabinoids has not been studied as a sleep-promoting agent in humans, theoretically, this could act as a thermoregulatory signal for circadian onset of sleep.

Sleep and Cannabinoid Use

Cannabinoid administration can alter sleep structure, partially dependent on the length of time one chronically uses. In the short term (<1 month), cannabis consumption is generally associated with desirable effects, including decreased sleep latency (time it takes to fall asleep), decreased wake after sleep onset (sleep disturbances), increased slow wave sleep (SWS) and reduced rapid eye movement (REM) sleep (Dedhia & Kole, 2021). Certain clinical populations benefit from decreased REM sleep, such as those with Post-Traumatic Stress Disorder (PTSD) who desire a reduction in sleep disturbances and in nightmares, a characteristic coinciding with less REM episodes (Bonn-Miller 2014). Animal and human studies examining long-term use describe a reduction in SWS sleep, decreased REM sleep and increased latency to the first REM episode, increased sleep disturbance, and decreased total sleep time, indicating that as tolerance to cannabis grows, adverse effects on sleep architecture build (Kaul et al., 2021). A reduction in SWS and in REM indicates that chronic users tend to spend more time in Stage 1 and Stage 2

sleep, which do not result in the same restorative outcomes as SWS or REM sleep stages (Dijk, 2009).

Cannabinoid ratio (THC:CBD) influences the sleep effects of cannabis with evidence indicating that THC is the primary sleep-promoting component. CBD products with minor amounts of THC have resulted in subjective improvements in sleep (Spindle et al., 2020). In high doses, isolated CBD can have wake-promoting effects, while THC in isolation typically promotes sleep in healthy individuals (Nicholson et al., 2004), also dependent on dosage. These findings imply that CBD is more likely to induce sleep in the presence of THC, although research is limited. Cannabinoids may also indirectly promote sleep by relieving pain, nausea, anxiety, spasms, and itching (Dedhia & Kole). For example, a study lead by Dr. Ethan Russo investigated the utility of a 1:1 THC to CBD ratio for pain-related sleep difficulties with success, demonstrating that cannabinoid breakdown may be instrumental in determining therapeutic effects (2007).

However, these benefits come with caveats should individuals continue regular cannabis use. Tolerance builds, requiring more THC to achieve the same desired sleep benefits (Halikas et al., 1985; Gorelick et al., 2013). Abstinence from cannabis use commonly results in adverse changes to sleep architecture, with the severity depending on a variety of factors including age of use onset and heaviness of use (Bolla et al., 2008; 2010). Cessation of use is associated with subjective reports of worsened sleep quality coupled with physiological measures finding decreases in total sleep time, slow wave sleep, REM sleep, and overall sleep efficiency. Sleep efficiency is an assessment of total time asleep against total time in bed. Studies also found increases in sleep latency, wake after sleep onset, and periodic limb movements (Bolla et al., 2008; 2010). Disturbed sleep

is cited as an impactful reason for returning to cannabis use in those who try to quit (Copersino et al., 2006; Budney et al., 2008).

Circadian and Homeostatic Attenuation of Cognition

Alertness and cognitive ability oscillate in conjunction with the 24-hour sleep-wake cycle. In healthy individuals, alertness peaks during daytime hours and reaches a low during nighttime. This reduction in alertness corresponds with behavioral markers (subjective tiredness) and physiological markers in the form of nighttime decrease in core body temperature and brain activity (Valdez, 2019). In addition to night-day variations in attention, research suggests that there may be time-of-day fluctuations dependent on individual chronotype (variations in circadian preference) and peak levels of arousal (Schmidt et al., 2007).

Homeostatic variables factor into cognitive performance. Attention capabilities are sensitive to sleep deprivation from the prior night and time of day (Schmidt et al., 2007). As the day progresses, levels of the neuromodulator adenosine accumulate, contributing to building sleep pressure. Adenosinergic tone significantly impacts arousal, vigilance, and subjective sleepiness in both rested and sleep-deprived people (Urry & Landolt, 2014). Sleep-deprived individuals experience sleep pressure buildup earlier in the day than those who slept adequately. Circadian rhythm, homeostatic sleep drive, and the interaction of the two primarily determine neurocognitive changes over the course of the day period (Carrier & Monk, 2000).

Cannabis and Cognition

Research on cannabis use and cognition has yielded inconclusive results. Long-term heavy use beginning in adolescence can lead to persistent deleterious neurocognitive effects in adulthood, although these effects may be reversible following cessation of use. A 2020 metanalysis analyzed 30 studies to determine the subacute cognitive effects of long-term (≥ 2 years) and regular cannabis use (≥4 days a week) in recreational users. Researchers found meaningful but minor decreases in cognitive performance in the domains of executive function, learning and memory, and global cognition; and moderate deficits in decision-making post intoxication (mean ≥12 hours). No meaningful differences were found between groups in attention, working memory, and information processing (Lovell et. al.). Another review converged findings from 5 metanalyses, encompassing 69 cross-sectional studies that investigated post-intoxicative cognitive performance. Chronic users most consistently performed worse compared to control groups on learning and memory-related tasks with effect sizes ranging from small to moderate. Small effect sizes were reported for deficits in attention, executive functioning, and processing efficiency (Bourque & Potvin, 2021).

A variety of factors, including early-onset use, frequency of use, and potency of cannabis may influence the degree of deficits (Kroon, et al., 2021). Longitudinal studies have found that early adolescent users tend to have verbal memory and attention deficits, and impairment is more likely to persist post-abstinence in adolescents than in adult users (Tait et al., 2011; Jacobus et al., 2009). Heavier use across age groups is associated with greater impairments compared to controls in global neuropsychological function assessments, including executive functions, attention, learning and memory, motor skills,

and verbal ability (Volkow et al., 2016). CBD is suggested to attenuate the adverse neuropsychological effects of THC (Lorenzetti et al., 2016), implying that cannabis potency affects degree of deficits. More research must be done to extricate the mechanisms behind cannabis effects on cognition.

Current Study

The aim of the current study is to investigate the replicability of Whitehurst et al.'s study, examining the biological rhythms, cognitive performance, and sleep patterns of young adult cannabis users and comparing them to age-matched controls. All participants were monitored over a two-week period using actigraphs and daily sleep/substance use journals. In alignment with the previous literature and with the original study, chronic users are expected to have better circadian entrainment and mild to moderate impairments in sleep and cognitive performance. Degree of impairment may depend on factors such as frequency of use and years of chronic use.

Method

Participants

Thirty-seven JMU students and Harrisonburg community members were recruited for the study via email (Appendix A) or word-of-mouth. One participant in the user group was excluded from analyses due to suspected circadian rhythm disorder, reducing the total participant sample to thirty-six (CB $\underline{n}$ = 18, M_{age} = 21, SD = 1.06; Control $\underline{n}$ = 18, M_{age} = 22, SD = 1.44). The total sample contained 24 females (CB = 9, Control = 14). Thirty participants identified as White/Caucasian, 3 identified as Multiple Races, 2

identified as African-American/Black, and 1 participant identified as Asian/Pacific Islander. Incentives included a raffle to win one of three gift cards equating to $50, $50, or $100, and a summary of sleep behaviors.

Materials

Inclusion Survey/Demographic Information. The inclusion survey screened potential participants for their age, sleep disorder diagnoses or disturbances, psychological disorder diagnoses, cannabis use history, and plans to change pattern of use in the next month. Exclusion criteria includes history of substance abuse and/or psychotic disorder, being below the age of 18, history of sleep disorder, desire to seek professional help for sleep disturbances, use of prescription sleep medication, using less than 5 days a week (for the cannabis group), and plans to reduce cannabis use in the next month. Once accepted to the study, participants were asked about demographic information, including race(s), gender/biological sex, academic level if JMU student, and how they heard about the study.

Cannabis Test Kit. THC Urinalysis screenings were conducted using STATDIP One-Step Dip sticks. The tests only indicated the presence or absence of THC metabolites in the urine sample, no other drug metabolites were detected. The cutoff for THC detection is 50ng/ml. Tests were used to confirm group membership, conducted at the first visit and the last visit to the lab. James Madison University guidelines for handling biomedical waste were followed.

Morningness-Eveningness Questionnaire (MEQ). The MEQ is a 19-item chronotype assessment designed by the Columbia University Medical Center meant to differentiate between "morning types" versus "evening types." With a possible range of 16-86, scores above 59 indicate "morning types," below 41 "morning types," and "intermediate types" falling between 42-58. A study performed in 2016 demonstrated good external validity of the questionnaire by comparing MEQ scores to actigraph results, with high correlation between the two (Natale et al, 2006). It was used to confirm that both groups kept comparable sleep-wake schedules. It was also used to determine if chronotype and cannabis use habits correlate, for example, if cannabis use behaviors are associated with an evening chronotype.

Pittsburgh Sleep Quality Index. The Pittsburgh Sleep Quality Index (PSQI) is a 19-item assessment tool used to inform one's subjective sleep quality. The items translate to 7 component scores measuring subjective sleep quality, latency, efficiency, sleep duration, sleep disturbances, habitual use of sleep medication, and daytime sleepiness which are then added to create a global PSQI score (Buysse et al., 1989). Scores range from 0-21 with 0 indicating high sleep quality and low sleep disturbance, and 21 indicating low sleep quality and high disturbance. A score greater than 5 is considered poor sleep quality. In a 2016 review, Mollayeva, et. al. found evidence of strong reliability and construct validity for the PSQI for both clinical and non-clinical samples.

Actigraph. The actigraph device is worn on one's non-dominant hand wrist and monitors the wearer's sleep-wake rhythm via computer program algorithms. Actigraphy

is traditionally used when researchers aim to assess circadian rhythm in a minimally invasive, natural sleep setting where polysomnography (PSG) would interfere (Martin & Hakim, 2011). While actigraphy is not a replacement for PSG recordings, studies have found the actigraphy software results are 91% accurate compared to PSG recordings (de Souza et al., 2003). At the initial intake session, participants who consent to the parameters of the study will be given an actigraph for the duration of their part in the study (2 weeks). Actigraph data will be used as a physiological measurement of sleep parameters and circadian rhythm compared between groups and in within group analyses.

Sleep/Use Diary. A diary is completed morning and night by participants. The morning version requests participants to self-report their sleep behaviors from the night prior, including when they woke up, time they got out of bed, how long they think it took them to fall asleep, how many times they remember waking up during the night, how many minutes they were awake at night, how refreshed they feel, and whether they woke up at that time for a commitment. The evening version asks participants to report on their cannabis use that day, other drug use, naps, and whether they took off the actigraph. Drug use other than cannabis will be reported, excluding daily prescription drugs because those are already reported in the demographic form.

Automated Neuropsychological Assessment Metric (ANAM). The ANAM is a non-diagnostic computer software tool created by the United States Army (2008) to measure baseline cognitive function prior to deployment. Studies have repeatedly found evidence supporting its reliability and construct validity (Kane et al., 2007; Short et al.,

2007). A battery created from the ANAM library comprised of four subtests was used to gather information on the participants' attention, memory, response inhibition, reaction time, and processing speed. The assessment takes about 20 minutes to complete. The cannabis user scores were compared to non-cannabis user scores in statistical analyses. Cannabis user data were also analyzed to investigate the predictability of years of use and heaviness of use in ANAM performance. Variables of interest in the current study include number of, and percent correct responses and reaction time measured in milliseconds. The ANAM was administered twice, one week apart, to buffer for technical difficulties or misinterpretation of the instructions in the first session. Scores will then be averaged out provided that the two sessions do not differ significantly.

Sleepiness Scale. Subjects report their subjective sleepiness using a set of statements about levels of sleepiness, ranging from "Feeling very alert, wide awake, and energetic" to "Very sleepy and cannot stay awake much longer." It is a revised version of the Stanford Sleepiness Scale. This scale is used to inform data analyses, investigating whether individual sleepiness and consequent alertness levels better predict ANAM reaction times and/or number of correct responses than other variables of interest.

Go-No-Go. The participant is presented with two characters ("x" and "o"). The user is instructed to respond as quickly as possible to the "x" by pressing a button each time the stimulus appears. When the "o" appears, the user must not press any key. This measures motor response inhibition. Differences between users and nonusers will be examined in addition to within group analyses based on history and frequency of use.

Memory Search. Participants are presented with a set of letters to memorize. They are then shown one letter at a time and must press a designated key to indicate whether or not the letter was in the original set. It is used to assess verbal working memory, immediate recognition, and attention.

Simple Reaction Time. Participants are told to respond as quickly as possible to one symbol shown repeatedly on the screen. As a measurement of simple reaction time, it assesses visuo-motor response, alertness, and vigilance.

Procedural Reaction Time (Basic Block). Participants must differentiate between "low" and "high" numbers displayed in a large dot matrix, with numbers ranging from 2-5. Low numbers are designated as 2-3 and high numbers are 4-5. It's used as an index of processing speed and reaction time.

Certificate of Confidentiality. This certificate was issued by the National Institute of Health (NIH), protecting participants' identifiable information from being released. With this, researchers can refuse to disclose personal information to "any civil, criminal, administrative, legislative, or other proceeding at the federal, state or local level." (NIH, 2019). Participants are given a description of the Certificate and a link to the NIH website detailing its purpose.

Procedure

Intake/First Session. Select criteria-meeting participants received an email inviting them into the Sleep and Pharmacology lab for an initial intake, where they were refreshed on the research protocol and goals of the study. All participants were then

tested for THC using urinalysis. They then complete surveys: the Morningness-Eveningness Questionnaire and Pittsburgh Sleep Quality Index; as well as a sheet of demographics questions. The first ANAM session was then completed. Researchers stepped out of the room while the ANAM was completed to reduce distractions. All ANAM sessions were completed between 9am-6pm. Lastly, participants signed up for their second lab visit scheduled for one week later.

Second Visit. Participants were asked if they've had any irritation with the actigraph device, take the ANAM again, and had their actigraph data downloaded to ensure the watch was working properly. They then scheduled a final visit for one week later.

Final Visit. Participants were drug tested once more to ensure cannabis use or lack thereof. They turned in the actigraph and were thanked for participating in the study. Individual sleep summaries are emailed to consenting participants at the end of data collection.

Results

Participants exhibited 97% compliancy in wearing their actigraph for their respective two-week period in the study. One participant wore the actigraph only at night, so their data could not be used in circadian analyses but was used when examining sleep parameters. Another participant admitted to smoking cannabis during the study, but tested negative on the subsequent urinalysis, so data were retained. All participants in the cannabis user group (CB) tested positive for THC on both urinalyses. Participants in the cannabis user group had been consuming cannabis chronically for 3 years (SD = 1.97) on

average, typically beginning to use at the age of 18 (SD = 1.06). Users reported consuming cannabis an estimated average of 96.67 (SD = 46.23) times over the two weeks in the study, operationalized as the amount of "hits" or "puffs" they took across sessions (see Table 2 for frequency count of hits by participant). All cannabis users reported smoking/inhalation as their main method of administration. Two participants reported using a vape, all others reported using pipes/joints/similar apparatuses, and 2 consumed edibles in addition to smoking. Other drug use was reported during the duration of the study (Table 3). For the MEQ, both groups scored in the intermediate chronotype range, indicating similar preferred sleep-wake times between groups. Across groups, participant did not differ significantly in average PSQI scores (CB = 6.67, SD = 4.06; Comparison = 7.44, SD = 4.12), or MEQ scores (CB = 50.22, SD = 8.1; Comparison = 51.61, SD = 9.5). A PSQI score above five indicates an individual may have poor sleep quality. Within the cannabis group, greater years of chronic use positively correlated with PSQI score (r = .48, p = .023). To further explore this relationship, a simple regression was conducted, to investigate the predictability of years of use for PSQI score. Years of chronic use predicted up to 29% of the variance in PSQI score [R^2 = .23, $F(1,16) = 4.715$, p = .045], indicating that in this sample, longer years of chronic use may be associated with worsened subjective sleep quality.

Sleep

No significant between-group differences were derived from sleep parameters. Independent sample t-tests and effect sizes were calculated for each sleep measure (see Table 4 for complete list of sleep parameters). During the week, the CB group tended to

spend more time in bed in bed (M = 490.46 minutes, SD = 53.76) compared to comparison group (M = 473.18 minutes, SD = 43.18), [$t(34) = 1.06{,}3$ $p = .295$, $d = .35$]. The CB group also displayed trends for later average wake time (M = 8:16 AM, SD = 67 minutes) than Comparison (M = 7:37 AM, SD = 72 minutes), [$t(34) = 1.7$, $p = .098$, $d = .57$]. These effect sizes indicate that a moderate difference may exist between groups in total time in bed and weekday wake-time. No trends were observed for weekend sleep behaviors.

Within the cannabis group, frequency of use positively correlated with minutes spent awake in bed ($r = .63$, $p = .002$) and negatively correlated with sleep efficiency ($r = -.58$, $p = .006$). Simple regression analyses were performed to further examine these relationships. A model containing frequency of use was used to predict sleep efficiency, which is a measure of total time spent asleep in bed as opposed to time spent awake while in bed. The overall model was meaningful [$F(2,15) = 4.046$, $p = .039$], accounting for 35% of the variance in sleep efficiency ($R^2 = .35$). As shown by the negative slope and squared correlation coefficient, more frequent use is associated with decreased sleep efficiency. The same model predicted minutes spent awake in bed [$F(2,15) = 5.231$, $p = .019$] and accounted for 41% of the variance ($R^2 = .41$).

Circadian Rhythm

No significant differences were found between groups on circadian entrainment analysis. The average fit to the circadian cosine model for the CB group ($R = .56$) did not differ meaningfully from the Comparison group ($R = .54$), [$t(27) = .526$, $p = .603$], indicating both groups are similar in entrainment. Correlational analyses were run to

determine the strength of circadian entrainment to the 24-hour model for both groups, but the CB group (r = .37) and Comparison group (r = .42) did not differ significantly, [$t(25)$ = -.825, p = .419]. One participant's data were excluded from that analysis due to failure to follow daytime wearing instructions. Heavier cannabis use was correlated with decreased strength in circadian rhythm (r = -.44, p = .049). Regression analyses were run to investigate the predictive properties of use frequency in circadian entrainment but were insignificant for both cosine model fit and strength of rhythm.

Cognition

Paired sample t-tests were completed to determine differences between session 1 and session 2 of the ANAM. Significant differences were found between sessions on the procedural reaction time task, (session 1 = 621.27, SD = 46.84; session 2 = 560.5, SD = 52.5), [t(35) = 5.55, p < .001, d = 1.3]. Accordingly, sessions from this task were left separate (session 1 and session 2) for all analyses. All other task session reaction times and correct response counts were averaged for further analyses. No significant differences were found between groups on an ANAM task; however, one trend was observed in mean reaction time. On average, the CB group showed a trend for a slightly slower mean reaction time in the Memory Search task (M = 919.98, SD = 232.43) than the Comparison group (M = 806.25, SD = 188.11), [$t(34)$ = 1.614, p = .058, d =.53]. See Table 5 for complete ANAM results.

Within the cannabis group, frequency of use positively correlated with average memory reaction time (r = .61, p = .004) and with average Go-No-Go reaction time (r = .50, p = .018). Years of use positively correlated with average Memory Search reaction

time (r = .49, p = .02). No correlations were found for correct response patterns. Sleepiness scale ratings also did not significantly correlate with any ANAM outcomes. A multiple regression model was used to further investigate the relationship between usage patterns and ANAM tasks. A model containing frequency of use and years of use significantly predicted reaction time for the Memory Search task [R^2 = .57, F(2,15) = 9.748, p = .002]. Their interaction fell just above the line of statistical significance, [F_{change}(1,14) = 4.233, p = .056]. Both frequency of use [b = 3.36, p = .004, sr^2 = .34] and years of use [b = 60.83, p = .02, sr^2 = .19] significantly contributed to the model, accounting for 19% and 34% of the variance, respectively. As evinced by the positive slope and squared semipartial correlation coefficient, higher frequency of use and years of use were associated with longer reaction time in the Memory Search task. A simple regression investigating the predictive utility of frequency of use significantly predicted Go-No-Go reaction [R^2 = .25, F(1, 16) = 5.302 p = .035]. Higher frequency of use was associated with longer reaction time in the Go-No-Go task.

Discussion

The aim of this study was to replicate Whitehurst et al.'s 2015 study, however, due to differences in recruitment, a slightly different sample was used here. The original study recruited a sample with greater average years of use, younger average age of chronic use onset, and lower reported frequency of use than the current sample. In contrast, the current study did not find meaningful differences between groups in sleep parameters, circadian rhythm entrainment, or cognition between chronic users who had been using chronically for an average of 3 years and non-users. However, this study

provided limited evidence that years of use and frequency of use may have some predictive power for certain sleep, circadian, and cognitive outcomes.

In the current study, no meaningful differences were identified between groups in sleep parameters. Several trends were observed, both in average wake-time and in total time in bed, with the cannabis group having a later average weekday wake-time and longer average duration in bed. While the effect sizes were moderate, the standard deviations were large for both variables, suggesting the variance was too great to distinguish meaningful differences. Increasing the sample size and thereby the statistical power may also aid in determining any meaningful differences. Although the mean hours of time in bed and wake-times were within 40 minutes of each other, even a 25-minute difference in wake-time can impact an adolescent's school performance the following day (Wolfson & Carskadon, 1998). Similar to the current study, Whitehurst et al. found a trend for later wake-times in the cannabis group. The current study found no other between-group differences, while the Whitehurst et al. study found that the cannabis user group had significantly greater sleep disturbances compared to non-users.

The current study found some potential evidence of predictive qualities in years of use and frequency of use. Higher years of chronic use positively correlated with higher PSQI, suggesting worsened subjective sleep quality may be associated with long-standing use. This is not to say more years of use cause worsened sleep quality; this is just an associative relationship. Higher reported frequency of use during the study was associated with lessened sleep efficiency, suggesting heavier users in the study spent more time in bed awake than lighter users; however, it is important to note that none of the cannabis users fell below a sleep efficiency of 85%. To be considered healthy, sleep

efficiency should be at least 80-85% (Shrivastava et al., 2014). Correspondingly, higher frequency of use also accounted for part of the variance in minutes spent awake in bed, which aligns with recent literature. Limited research has found evidence for early-onset cannabis use to result in shorter sleep duration in adulthood (Winiger et al., 2019; 2020). Longitudinal studies sampling the adult population have found shorter sleep duration among chronic users, but do not report differences amongst early onset users and those who started later-in-life (Diep et al., 2022).

The current study did not find meaningful differences in between groups in circadian entrainment or strength of rhythmicity. Whitehurst et al. found that cannabis users had significantly better circadian entrainment to the cosine model, as well as stronger rhythmicity to the 24-hour cycle. This is suspected to be due to the difference in cannabis group samples. Given that our sample had decreased years of chronic use, a later average age of onset, and an estimated more frequent use in a shorter period of time, these variables may influence circadian entrainment in chronic users. To the author's knowledge, no prior research is devoted to the effects of early adolescent cannabis use on later-in-life circadian rhythm; however, studies have investigated its structural and functional effects on the adult brain. Adolescence is a crucial time for neurodevelopment, including the maturation of the endocannabinoid system, making the ECS susceptible to exogenous perturbations (Schneider, 2008). Repeated exposure to cannabis during youth may alter the course of brain maturation in a way that modifies circadian functioning.

Similar to the sleep and circadian results, significant differences were not identified between groups in cognitive task reaction time, nor in number of correct responses on each task. The cannabis group had a moderately slower reaction time in the

Memory Search task, however as this was measured in milliseconds, this difference is likely unnoticeable to the individual. One limitation discussed in Whitehurst et al.'s study was the potential for participants to be acutely intoxicated during the ANAM, meaning their results were due to acute rather than subacute effects. Based on self-report in evening sleep journals, no cannabis user in the current study reported a session with three hours of their scheduled ANAM, implying acute effects did not interfere with performance. The current study did not include the Code Substitution (CSI) task, a measure used as an index of immediate and delayed memory, due to technical difficulties administering the task; however, working memory was still assessed in this study through the Memory Search task. Between-group trends were found in the original study sans statistical significance in the CSI task, with the cannabis group have a slower average reaction time than comparison. The original study also observed higher frequency of use negatively correlating with percent correct on the CSI task and number of correct responses on the Go-No-Go task. The current study found no such patterns but found that higher frequency of use and higher years of use predicted longer reaction times for the Memory Search task. The lack of effect these predictors had on correct responses implies that they may have impacted processing efficiency and visuo-motor response related to working memory. Higher frequency of reported use predicted longer Go-No-Go reaction time but not number of correct responses, suggesting heavier use may interfere with visuo-motor response time and processing efficiency related to response inhibition.

These findings align with previous reports of dose-related cognitive deficits within chronic cannabis user groups. Heavy use has been associated with marked cognitive deficits even after 28 days of abstinence (Bolla et al., 2002) and recent

metanalyses have found consistent effect sizes for learning and memory deficits in chronic users (Bourque & Potvin, 2021; Figueiredo et al., 2020). Heavy use is concurrently associated with structural and functional changes in brain areas densely innervated with CB1 receptors, including the hippocampus, prefrontal cortex, cerebellum, and amygdala. Earlier age of onset, higher frequency of use, and potency (greater amounts of THC to CBD) contributed to these changes CBD may protect against adverse cognitive alterations associated with THC (Lorenzetti et al., 2016). Functional changes have been observed in attention and working memory-related neural activity, resulting in recruitment of other brain areas to augment deficits (Weinstein et al., 2016). However, all metanalyses note that a major limitation in cannabis and cognition research is lack of methodological consistency.

The ANAM was chosen as the cognitive assessment for this study due to its evidenced reliability. It was designed to minimize practice effects and has amassed evidence in doing so (Vincent et al., 2018; Roebuck-Spencer et al., 2007). However, Gilmore et al. found numerous significant differences between two ANAM administrations performed two weeks apart in a sample containing university students (2021). The current study found significant differences between administrations only in the Procedural Reaction Time task, with participants performing faster the second time. Similar to Gilmore et al.'s sample, current participants may have felt more comfortable with task instructions the second time around. Retrospectively, it would have been beneficial to incorporate the ANAM Mood Scale as an index of test anxiety. This would be used in analyses to determine whether any between- or within-group differences could be better explained by subjective anxiety levels relating to being tested.

One limitation of this study was its small sample size. A larger, more representative sample may allow for more inferences to be made regarding both the between-group and within-group results. This study was also limited to observing users' preestablished behaviors and usage patterns, resulting in route of administration variance and lack of control over cannabinoid ratio. All but two participants reported "smoking/inhalation" as their only method of administration over the duration of the study, reporting the amount of hits/time of sessions daily. Additionally, not all hits result in the same amount of THC released; for example, one hit of a bong is more potent than one hit of a pipe or joint. All participants varied in the apparatuses used (i.e., pipes, joints, bowls), sometimes from session to session. Beyond differences in bioavailability between apparatuses, the amount of THC inhaled also depends on the smoking dynamic of the individual user and can range in bioavailability from 2-56% (Huestis, 2009).

The two users who reported consuming edibles in conjunction with smoking were asked to report how many mg of THC consumed, as this was the most accurate way of defining dosage. Ingesting cannabis results in a different pharmacokinetic profile than inhaling. When ingested, onset of psychoactive effects is delayed, and clinical effects can last up to 12 hours. A higher concentration of 11-hydroxy-delta-9-tetrahydrocannabinol is released in the body (11-Hydroxy-THC), the main active metabolite of THC. When inhaled, onset of psychoactive effects occurs more rapidly and last up to 4 hours (Oberbarnscheidt & Miller, 2016). Participants were also asked to report the cannabis ratio or product used if they could, but not enough participants knew that information for every session to evaluate differences. It was consequently difficult to operationalize the

precise amount of THC that participants consumed through inhalation methods and compare across all cannabis-using subjects.

The consumption of psychoactive substances besides cannabis may have served as another limitation (Table 3 shows number of substances by participant group). Reported alcohol use was restricted to Thursday-Saturday for all but one subject who reported alcohol use every day. Given the low amount of reported alcohol and nicotine use in a largely undergraduate student sample, several participants were verbally asked at their last session if they had used alcohol and if they had reported it. These students had used alcohol during their time in the study but hadn't thought to report it as another "drug or supplement." The lack of reported extraneous substance use limits our interpretation of weekend data and could significantly impact average fit to the cosine model and strength of rhythm. Alcohol use, especially binge drinking, has been demonstrated to acutely disturb circadian rhythmicity and sleep homeostasis (Hasler & Pederson, 2020).

Future Directions

This study intended to replicate Whitehurst et al.'s study, however, a slightly different sample was selected. Based on differences between our cannabis group and the original study, we may have identified key predictive factors in the effects of cannabis on circadian rhythm. A later mean age of onset, less years of chronic use, and heavier use likely contributed to this study finding equivalent circadian entrainment and strength to the model between-groups. While this study is small in scope and assessed a college-age population, the patterns identified in this study should be explored further in a larger, non-student population. Estimated frequency of use reported during the study predicted

multiple variables of interest in both sleep and cognitive domains. Dose-dependent differences between light, moderate, and heavy users in sleep, circadian, and cognitive parameters warrant continued exploration, as do the predictive properties of early age of onset and years of use.

Controlling dosage, cannabinoid ratio, and method of administration should be incorporated in future studies. Differences between cannabis users who exclusively inhale and those who exclusively ingest cannabis should be investigated and should also be compared with a non-user group. Future studies should also restrict usage of other psychoactive substances to minimize confounds. An anxiety scale should be added to the ANAM battery to determine whether test anxiety served as a performance predictor.

Table 1.

Demographics

	N	**Gender**	**Age**
CB	18	9 (F)	21(1.06)
Comparison	18	14 (F)	22(1.44)
Total	36	24 (F)	21(1.45)

Standard deviation for age shown in years

Table 2.

Total reported use during study by participant

Participant	Total Number of Reported "Hits"
1	30
2	35
3	44
4	46
5	48
6	66
7	79
8	84
9	100
10	104
11	104
12	109
13	123
14	127
15	132
16	148
17	165
18	189

The total number of "hits" column indicates the reported number of times a participant took a "hit" of cannabis during their time in the study

Table 3.

Participant Drug Use by Group

	Alcohol	**Caffeine**	**Nicotine**	**OTC**	**Prescription**
CB	4	15	2	4	6
Comparison	3	11	--	4	9

Note: Values indicate the number of participants using each drug

Table 4.

Sleep Parameters by Group

	Sleep Onset Time	**Wake Time**	**Total Sleep Time (min)**	**Sleep Latency (min)**	**Sleep Efficiency %**	**WASO (min)**
CB	24:26(63)	8:16AM(67)	490.46(53.76)	15.6(7.57)	92.07(3.45)	28.02(23.3)
Comparison	24:04(54)	7:37AM(72)	473.18(43.18)	20.58(18.6)	91.8(6.9)	23(14.66)

Standard deviations shown in parentheses. All standard deviations are displayed in minutes except for Sleep Efficiency, which is in percentage points.

Table 5.

ANAM Performance by Group

Metric	CB	Comparison
Memory Search reaction time	919.98(232.43)	806.25(188.11)
Memory Search # correct	35.2(6.08)	36.07(4.07)
Memory Search % correct	88(15.2)	91.67(10.16)
Simple reaction time	295.59(26.6)	287.1(22.3)
Go-No-Go reaction time	349(18.51)	350.84(18.63)
Go-No-Go # correct	113.75(3.17)	112.21(4.88)
Go-No-Go % correct	90.22(16.43)	94.75(8.33)
Procedural reaction time (1)	621.27(46.85)	580.7(58.43)
Procedural reaction time (2)	560.5(52.8)	562.04(70.17)

Mean reaction times depicted in milliseconds for memory search, simple reaction time, Go-No-Go, and the two procedural reaction time sessions. Number and percent correct shown for memory search and Go-No-Go tasks. Standard deviations displayed in parentheses.

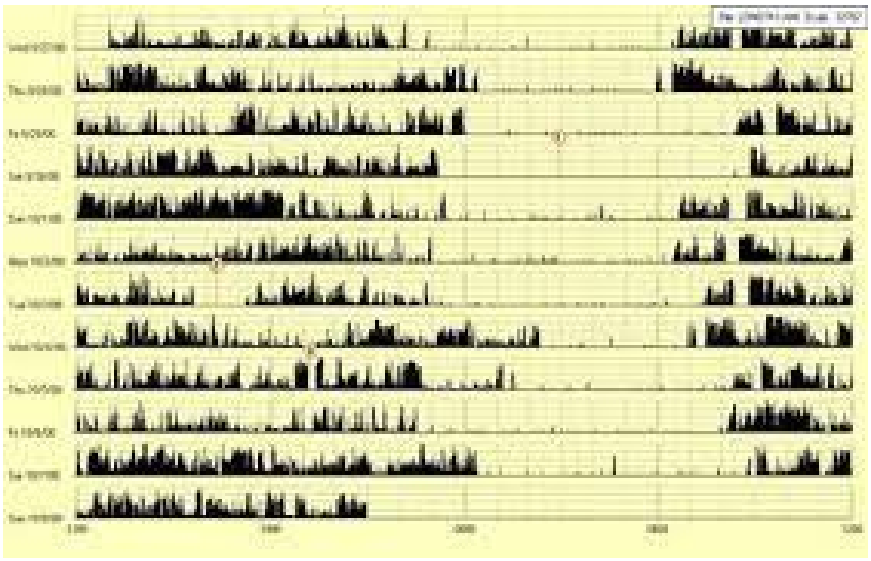

Figure 1. Example of actigraph data.

Appendix A

Participant Email

“Hello!

We are seeking both daily cannabis users and non-users to participate in a sleep & circadian rhythm study at JMU. Complete our anonymous survey (linked below) to find out if you are eligible. If so you’ll be contacted with more details about the study. Upon participation you’ll have the opportunity to win one of three Visa gift cards ($50, $50, $100, respectively) and receive an analysis of your sleep patterns.

This study has been approved by the IRB, protocol # 22-2873.”

Appendix B

Screener Survey

Participant ID: _____

1. Please enter your age.

_____

2. Are you a full-time JMU student (Yes/NO)

3. Have you been diagnosed with any of the following?

__ Mood disorder

__ Personality disorder

__ Neurological disorder

__ Anxiety disorder

__ Psychotic disorder

__ Trauma-related disorder

__ Substance use disorder

__ Other

__ No

4. Have you been diagnosed with a sleep disorder? (Yes/No)

5. In the past month, have you considered seeking medical help for sleep disturbances? (Yes/No)

6. Have you ever used cannabis (Delta-9-THC)? (Yes/No)

7. Have you used cannabis (Delta-9-THC) in the past month? (Yes/No)

8. Approximately how many days a week do you use cannabis?

_____

9. For how long (in years) have you been a habitual cannabis user?

_____

10. Do you plan on changing your cannabis use patterns within the next month?

IE, reducing or increasing your use?

10a. If yes, please elaborate:

_____

11. Please provide your email address if you are interested in participating in the study. It does not have to be your JMU email address. *If you do not provide an email address, your response will NOT be recorded*

_____

Appendix C

Sleep Journal

Participant ID: _____

1. Did you take any naps today? (Yes/No)

    1a. If yes: At what time: _____ 1b. How many hours/minutes: _____

2. Did you take off the actigraph at any point today? (Yes/No)

    2a. For how long: _____

3. Did you consume cannabis today? (Yes/No)

    3a. If yes – in what form? Check all that apply.

        __ Edibles

        __ Smoking (blunts/pipes)

        __ Vapes

    3b. If edibles – how many milligrams THC consumed/what strain?

        _____

    3c. If smoking – how many hits/sessions? If you know the strain please indicate that or write N/A. _____

    3d. At what time(s) approximately?

4. Was there anything unusual about your day that caused you to be more stressed or excited?

5. Did you consume any caffeine today? (Yes/No)

    5a. How many milligrams or cups? _____

6. Did you consume any other drugs or supplements today (besides prescription medications)?

6a. If yes, please list:

_____

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