

Spring 2013

Effects of chronic marijuana use on circadian rhythms, sleep, and cognitive performance

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Effects of Chronic Marijuana Use on Circadian Rhythms, Sleep, and Cognitive
Performance

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A thesis submitted to the Graduate Faculty of

JAMES MADISON UNIVERSITY

In

Partial Fulfillment of the Requirements

for the degree of

Master of Arts

Psychological Sciences

May 2013

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Abstract

Marijuana use has been linked to various circadian related activities like sleep and cognitive performance (Bolla et al., 2002; 2008; Budney et al., 2002; Cohen-Zion et al., 2010; Iverson et al., 2005; Meier et al., 2012; NIDA, 2012; Pope & Yurgulenn-Todd, 1996; WHO, 2013). Animal literature suggests a connection between marijuana use and altered circadian rhythms; however, the effect has not yet been studied in humans (Acuna-Goycolea et al., 2010; Sanford et al., 2008). The present study seeks to examine the effect of chronic marijuana use on circadian function in humans and extend the knowledge surrounding marijuana's effect on neurocognition and sleep quality. Participants consisted of chronic marijuana users and age-matched non-drug user controls. Participant substance use was verified through urine samples obtained at the initial and final visits. Participants wore actigraphs and maintained sleep diaries for 3 weeks and both marijuana users and non-users took the Automated Neuropsychological Assessment Metrics (ANAM) to measure cognitive performance. Data analyses reveal chronic marijuana use may act as a zeitgeber and lead to increased entrainment in human users, however use may also result in slight cognitive impairment and significant sleep disturbance.

Effects of Chronic Marijuana Use on Circadian Rhythms, Sleep, and Cognitive Function

Most individuals engage a certain degree of rhythmicity with their daily environments. The majority wake, eat, drink and go to sleep at similar times each day. These cyclic and predictive behaviors are due to biologically and environmental determined circadian rhythms. Circadian rhythms are mostly endogenous daily oscillations that generally follow an approximate 24-hour cycle (Hasler & Clark, 2013; Wager-Smith & Kay, 2000). While there are over 100 examples of circadian oscillations, the sleep-wake rhythm is one of the most robust examples and as a result one of the most documented.

Although these rhythms are largely biologically driven, circadian rhythms can be disrupted by environmental factors. Jet lag and shift-work disorders are more common forms of circadian disruption; however, recently researchers have discovered that chronic administration of drugs of abuse have implications in circadian alterations (Falcon & McClung, 2009; Kolla & Auger, 2011; Kosubud et al., 2007). Marijuana, the most commonly abused illicit drug in the world (World Health Organization, 2012) has been shown to disrupt circadian-related activities such as neurocognitive functioning and sleep quality (Bolla et al., 2002; 2008; Cohen-Zion et al., 2010; Meier et al., 2012; Pope & Yurgulen, 1996). However, to my knowledge, no research has focused on chronic marijuana use and human circadian entrainment (see these cites for information on animals and circadian entrainment: Acuna-Goyocolea et al., 2010; Sanford et al., 2008; Whitmann et al., 2007). This paper will focus specifically on the effect of chronic marijuana use on human circadian

entrainment, by monitoring sleep-wake rhythms, as well as further the knowledge regarding marijuana's effect on neurocognition and overall sleep quality.

Circadian Rhythms

Circadian rhythms are maintained by behavioral constraints of our environment as well as endogenous, neural correlates. The suprachiasmatic nucleus (SCN), or the "internal clock," located in the anterior hypothalamus of the mammalian brain, is the endogenous control center for circadian rhythms. Lesions to rat SCNs have resulted in abolishment of a range of circadian behaviors, including sleep-wake cycles (Eastman, 1984; Ibuka & Kawamura, 1975; Saper et al., 2005). However, chronic administrations of drugs of abuse and consistent feeding schedules have been shown to restore circadian rhythmicity in animals with ablated SCN's (Iijima et al, 2002; Kosobud et al., 2007; Marchant & Mistlberger, 1997). This suggests that while the SCN is the primary center for rhythm production, other SCN-independent pacemakers may assist in circadian pattern generation (Iijima, 2002; Masubuchi et al., 2000).

The SCN has projections to other areas in the hypothalamus including the subparaventricular zone (SPZ) and the dorsomedial nucleus (DMH) (Saper, 2005). These projections allow for the SCN to communicate rhythm information to other areas in the brain like the ventral lateral preoptic nucleus (VLPO), a major structure in homeostatic sleep promotion (Saper, 2005; Siegal, 2004). This communication is facilitated by internal chemical messengers that assist in sleep-wake rhythm maintenance; for example, melatonin, a neurohormone produced in the pineal gland,

assists by acting with light to hold circadian rhythms to the 24-hour cycle (Liu et al, 1997). Additionally, other endogenous messengers include the neurotransmitters histamine, serotonin, acetylcholine, and gamma amino butyric acid (GABA) which all have been implicated in sleep promotion as well as synchronicity (Reghunandan & Reghunandan, 2006). In sum, the molecular and neurochemical design of the SCN allows organisms to anticipate daily environmental fluctuations instead of simply responding to environmental stimuli (Darlington et al, 1998). And while the internal environment is extremely important in circadian cycles, external cues, commonly called zeitgebers (time givers), also have their place in maintaining mammalian synchronization.

Zeitgebers and Dysfunction of Rhythms

The SCN combines various zeitgebers with efforts of internal messengers to promote consistent rhythmicity (Hasler & Clark, 2013). The most powerful zeitgeber is light, with others including such things as feeding, exercise, and social interaction (Hasler & Clark, 2013). These external cues help induce changes in the internal system to maintain consistency. The combination of the SCN, internal hormones/neurotransmitters, and environmental zeitgebers allow for individuals' circadian rhythms to reset daily, or to entrain to the 24-hour day (Liu et al., 1997; Saper et al, 2005).

Individuals may vary in degree of circadian clock entrainment. An individual's chronotype, or preferred sleep-wake schedule, can be in misalignment with their internal clocks' demands, resulting in circadian dysfunction (Hasler &

Clark, 2013). Circadian dysfunction or misalignment is a mismatch of circadian timing and the normal 24-hour rhythm (Sack et al., 2007). Circadian misalignment can show in various forms, some of the most common being phase advancement, phase delay, irregular rhythm, and non-24-hour rhythm. Phase advanced individuals, behaviorally, are active early in the morning and not as active in the evening. Phase delayed individuals are usually more active late into the night and not as active in morning hours. Irregular rhythms are characterized by normal total sleep times but varying times of sleep and wakefulness throughout the 24-hour day; while non-24-hour disorders are characterized by continuous movement of peak alertness (Sack et al., 2007).

Dysfunctions like phase advancement, phase delay, and irregularity have consequences for other circadian systems including but not limited to digestion, metabolism, and hormone regulation (Academy of Sleep Medicine, 2008; Laposky et al., 2008) Excessive sleepiness, gastrointestinal dysfunction, and irritable mood are all negative consequences of a disrupted circadian rhythm. Previous research has focused on misalignment in the form of shift work or jet lag as mentioned (Sack et al., 2007); however, recently, animal literature has implicated drugs of abuse in altered circadian entrainment (Falcon & McClung, 2008; Iijima et al., 2002; Kosobud et al., 2007) While majority of the work has focused on chronic stimulant use, like cocaine and amphetamine, a few animal researchers have also investigated chronic marijuana use and its effect on circadian rhythmicity (Acuna-Goyocolea et al., 2010; Sanford et al., 2008). While there has not been any research exploring the effect marijuana has on human entrainment, chronic users, often report using marijuana to alleviate sleep

difficulties (Bolla et al., 2008; Bonn-Miller et al., 2010). Additionally, one of the highest reported reasons for relapse among marijuana users is sleep initiation and maintenance (Bonn-Miller et al., 2010). Considering sleep-wake rhythms are a product of circadian processes, one would intuit an underlying relationship between marijuana use and circadian rhythms as well.

Marijuana

Marijuana, or cannabis, has become the world's most widely used illicit drug with 2.5% of the world's population reporting use, exceeding the prevalence of use for both cocaine, (.2%) and opiates (.2%) (WHO, 2013). Although criminalized in 1937, marijuana is also recognized as the most commonly abused illicit drug in America, with about 18 million Americans reporting regular use (National Institute on Drug Abuse, 2012) and the incidence of use is increasing. Marijuana use has increased 5.8% since 2007, unlike the consumption of most other drug substances, which have either decreased or remained constant (NIDA, 2012).

A plant of the genus, *Cannibus*, marijuana breaks down into various cannabinoids, most notably delta-9-tetrahydrocannabinol (THC). Cannabinoids are also produced in the mammalian system and two receptors for endogenous and exogenous ligands have been found to date, CB1 (Matsuda et al., 1990) and CB2 (Munro et al., 1993) Only CB1 is found in the central nervous system (CNS) while CB2 has been discovered in peripheral tissues and may have a significant function in immunoregulation (Felder & Glass, 1998). Notwithstanding the relatively recent discovery of the CB receptors, the endocannabinoid systems are the most ubiquitous

G-protein-coupled receptors found in the CNS and their abundance rivals that of ionotropic glutamate receptors; arguably the most well-known and widespread CNS receptors (Breivogel & Childers, 1998). Location of CB1 receptors have been mapped across the CNS and their presence has been isolated in the hippocampus, amygdala, thalamus, cerebellum, and spinal cord among other structures (Breivogel & Childers, 1998). THC, the most commonly studied exogenous cannabinoid, binds to the CB1 receptor, creating the psychoactive “high” from ingesting marijuana (Ashton, 2001; Hall & Solowij, 1998).

The psychoactive properties of cannabis include euphoric relaxation, perceptual alteration, time distortion, and intensification of sensory experience within a few minutes after consumption (Ashton, 2001; Hall & Solowij, 1998). The effect is prolonged due to the continued absorption of the drug from the stomach into the bloodstream (Ashton, 2001). Exogenous cannabinoids, like marijuana, are extremely lipid soluble and accumulate in fatty tissues reaching peak concentrations 4-5 days after ingestion (Ashton, 2001). The liver metabolizes cannabis and excretes cannabis metabolites into the stomach where they are reabsorbed into the bloodstream or as urine (Ashton, 2001), thus explaining why the most common way to measure cannabis use is through urinalysis.

Cannabinoid pharmacodynamics has also been recently described. Cannabinoids act as second messengers, modulating the release of other neurotransmitters through either an inhibitory action or excitatory action (Christie & Vaughn, 2001). For example, cannabinoids increase the release of dopamine from nucleus accumbens and prefrontal cortex neurons fortifying marijuana’s addictive

nature and exacerbating addictive, reinforcing properties of other drugs of abuse (Ashton, 2001; Hall & Solowij, 1998; NIDA, 2010). However, cannabinoids also act on GABA neurons, in which most cases, the excitation of endocannabinoids will result in a decreased release of GABA into the synapse. Depending on the action of GABA on the cells affected, the inhibition could have varying effects, such as decreasing the inhibitory action of GABA on the postsynaptic cell or releasing the cell from tonic inhibition furthering GABA's inhibitory action (Acuna-Goycolea, 2011; Ohno-Shosaku et al., 2001). Interestingly, the modulatory action of cannabinoids can be seen in areas of the CNS particular to circadian timing.

Marijuana and the SCN

Marijuana users have consistently self-reported time distortive effects of marijuana. Green, Kavanaugh, and Young (2003) examined 12 marijuana articles (all $N > 30$) and 71% of participants across all studies identified "slowing of time" as a primary effect of use. In addition, higher levels of intoxication were associated with reports of time alteration. Recently, sober marijuana users in Bolla et al. (2010) were described as spending more overall time in bed when compared to drug-free controls. As a result, scientists have begun to examine the SCN in animal models to further understand the time altering effects of marijuana use.

Immunoreactive staining of hypothalamic nuclei resulted in rich innervations of CB1 fibers (Wittmann et al., 2007). The presence of cannabinoid receptors in the hypothalamus, particularly the SCN, encouraged the search for an interaction between the CB1 receptors and external time cues. Researchers examined the presence of

cannabinoids in the hamster SCN and the interaction these receptors had with light. Sanford et al. (2008) confirmed earlier findings of CB receptors in the SCN and also extended cannabinoid receptor presence to the intergeniculate leaflet of the thalamus (IGL) and the dorsal and medial raphe nuclei, the first responsible for shifts in the circadian clock and the latter known to inhibit photic information relayed from the retina. Further, researchers found that when paired with light pulses, injections of CB1 receptor agonists produced phase advancement in hamsters. This provided evidence that cannabinoids may have a role in normal functioning of the mammalian clock and alterations in cannabinoids may also lead to circadian disruptions (Sanford et al., 2008).

Following Sanford et al. (2008), Acuna-Goycolea and colleagues (2010) further explored the interaction between CB1 receptors, SCN neurons, and light. These researchers hypothesized that the excitement in the SCN was not primarily due to the release of cannabinoids but due specifically to cannabinoid modulation of GABA release. When researchers monitored the effect of a cannabinoid agonist in the presence of light stimulation, they recorded an increased activity of the SCN and a phase advancement of the mice rhythms, similar to that of Sanford et al., but the same relationship in the presence of a GABA_A receptor blocker did not result in an increase in spike frequency of the SCN neurons or a phase advancement. With that, Acuna-Goycolea et al. (2010) determined cannabinoids do alter the ability of the SCN to entrain to environmental light cues, but mainly through modulating GABAergic tone. Taken together, these studies suggest the subjective time distorting effects of marijuana may have neural roots in the SCN and, in turn, chronic users of the

substance may possess circadian alterations. While the impact of cannabinoids and circadian function has been demonstrated in animal models, to my knowledge, the circadian effects of marijuana have not been measured in human users. However, there are studies on humans where the impact of Cannabis on sleep has been evaluated.

Sleep and Marijuana

Sleep is one of the most basic biological drives of all living animals. Sleep is a very persistent drive and will persist into wake states if individuals are not adequately rested (Rosenthal, 1998). Indeed, between 50 and 70 million Americans report suffering from a sleep problem (CDC, 2013). Sleep disturbance is also significantly correlated with overall psychological well-being (Steptoe et al., 2008). In fact, many who report sleep difficulties tend to turn to drugs of abuse, like marijuana to cope with their sleep and other related symptoms (Bonn-Miller, 2010).

Sleep has traditionally been described in two parts, rapid eye movement (REM) and non-rapid eye movement (NREM). NREM is composed of three stages (N1 - N3) with N3 designated as slow-wave sleep (SWS) (National Sleep Foundation, 2011). SWS is associated with the rejuvenating, restorative qualities usually associated with sleep and rest while REM has been implicated in cognitive performance and learning (NSF, 2011). There is still much debate in the literature on the importance of REM versus NREM sleep and many questions have yet to be resolved.

Marijuana users have consistently reported using to help alleviate sleep related difficulties, however research has implicated chronic use may result in further sleep-related problems (Bolla et al., 2008; Bonn-Miller et al, 2010; NIDA, 2010). Researchers have shown sleep architectural changes of chronic users with results indicating significant reductions in REM and increases in SWS are common during use while the reverse are seen during withdrawal (Bolla et al., 2008; Cohen-Zion et al., 2010). Architectural changes in sleep can be detected at least three weeks after cessation in chronic users (Cohen-Zion et al., 2009). In addition, one of the most common self-reported reasons for return to use after a quit attempt are sleep related problems like insomnia (Budney et al., 2002). Past research has reported shorter total sleep times (TST) as well as poor overall sleep quality for individuals chronically using marijuana (Bolla et al., 2010; Budney et al., 2002; Haney et al., 2004). Sleep and substance use have been highly correlated with each other as well as ranges of other negative outcomes such as mental illness, injury, chronic disease and illness, as well as impaired memory, attention and cognitive performance (Center for Disease Control and Prevention, 2012; NIDA, 2012).

Marijuana and Neurocognition

Long-term use of cannabis at extremely high-intake levels (greater than 5000 times in a lifetime) has been associated with adverse psychosocial outcomes including lower educational attainment and cognitive deficits (Pope et al., 2001). Research suggests individuals who begin cannabis use in adolescence may suffer from increased neuropsychological decline in adult years when compared to non-users (Meier, 2012). Further, individuals who smoke at higher intake levels may experience

greater cognitive deficits when compared to lower intake users and non-users in areas of planning, organizing, problem solving, memory, skill application and learning (Bolla et al., 2002; Crean, Crane, & Mason, 2011; Pope & Yurgulen-Todd, 1996) However, other studies have shown that chronic cannabis use may not lead to long-term cognitive decline and the use-associated deficits described subside shortly after abstinence (Iverson, 2005; Pope et al., 2001; 2002).

Current Study

The current study intends to explore sleep-wake rhythms, sleep quality and neurocognitive functioning of human, chronic marijuana (MJ) users by monitoring non-treatment seeking, chronic cannabis users over a 21-day period with actigraphy and self-report sleep and use diaries. MJ users will be compared to a group of age-matched, non-using controls. Based on previous animal research, a significant alteration in circadian rhythmicity of chronic MJ users when compared with controls is expected. In alignment with previous human research, a decrease in total sleep time and sleep quality in chronic MJ users is expected when compared to controls as well as increased neurocognitive deficits among self-reported “heavy” chronic MJ users.

Method

Participants

Thirty participants (MJ = 17, $M_{age} = 21.47$ $SD = 1.70$; Control = 13, $M_{age} = 21.54$ $SD = 2.11$) were recruited either through a recruitment email (Appendix A) or word-of-mouth from James Madison University (JMU) and the surrounding community. (see Appendix C for demographic information). The sample was

comprised of 17 females (MJ = 9; Control = 8). In terms of ethnicity, 26 participants identified as White/European, 1 as Black/African-American, 1 as African, 1 as Hawaiian/Native Pacific Islander and 1 as “two or more of the above.” A \$150 blind raffle and a comprehensive sleep and circadian analysis were used as incentive for participation.

Materials

Intake/Demographics/Exit Survey. The intake form asked participants about previous psychological or sleep diagnoses, marijuana and other illicit drug use, and potential of changing or altering marijuana use patterns within the observed month. Intake forms detailed the required urine samples and asked for a signature to indicate participant compliance. Demographic information included participant age, race, and daily activity schedules. Information about prescription medications and supplements ingested were also obtained. Exit surveys asked participants amount of marijuana consumed throughout participation as well as provided an opportunity for individuals to request substance abuse counseling services.

Morningness-Eveningness Questionnaire (MEQ). Nineteen item questionnaire designed by the Columbia University Medical Center to help identify “evening-types” and “morning-types.” Scores range from 16-86 with scores below 41 indicating “evening types,” above 59 indicating “morning types,” and between 42 and 58 indicating “intermediate types.” We utilized this assessment in the current study to help identify correlations between substance use and preferential bedtimes and wake times.

Sleep/Use diaries. Diaries asked for subjective reports of bedtime, wake time, total sleep time as well as substance use, both marijuana and other illicit substances throughout the duration of three-week participation. Diaries did not request information about prescription medications due to the information provided on demographic sheets (see Appendix B).

Automated Neuropsychological Assessment Metrics (ANAM) Adapted by the United States Army (2008) as a non-diagnostic tool to establish baseline cognitive abilities of service members (<http://www.armymedicine.army.mil/r2d/aman.html>). It is now distributed exclusively by VistaLife Sciences and has been scientifically proven to provide healthcare professionals a way to assess cognitive change (vitalifesciences.com/anam-intro.html). The test is comprised of various test subtypes which have been used to measure participants' cognitive speed, accuracy, memory, and thinking abilities in a variety of clinical subgroups. The scales and batteries used in the ANAM have been deemed reliable and valid (Johnson et al., 2008; Kabat et al., 2001; Segalowitz, 2007). The ANAM will provide a cognitive comparison between the two naturally occurring sub-groups, substance users and non-users. All volunteers were tested on the ANAM between 11am and 6pm to minimize circadian effects.

Code Substitution (CS). Digit-symbol pairs are presented in a learning phase and then participants were asked to recall the correct pairs in an immediate (CSI) and delayed (CSD) memory phase. Used as a measure of attention and memory retention.

Go-No-Go. Two characters are presented (i.e. “x” and “o”).

Participants instructed to respond when one of the characters is presented and inhibit response when the other is displayed, measuring overall response inhibition.

Memory Search. Characters are presented for memorization. During testing, individual characters from the memorization period were presented and participants were instructed to delineate between a character present in the memory set and a character that was not. Used to determine differences in short-term memory between chronic users and non-users.

Simple Reaction Time. Participants instructed to respond as quickly as possible to a previously established stimulus to help establish between-group processing differences.

Procedural Reaction Time. Participants presented with a series of numbers (2-5) and instructed to differentiate between “high” numbers (4 or 5) and low numbers (2 or 3). Slower speeds are indicative of low processing efficiency.

MEDIMPEX Dip Strip THC Testing Kit. FDA approved marijuana test-strips were utilized to monitor THC-metabolite in participants urine on two occasions, intake and final visits. Tests had standardized cut-offs of 50ng/ml of THC-metabolite. Cut-off was utilized to determine group membership. All urine samples were collected in accordance with the biomedical safety guidelines outlined by James Madison University.

Actigraph. Reliable, objective measure of sleep and circadian rhythms, see Ancoli-Israeli (2003) for more information. Actigraphy data was initially analyzed

using Action-W Version 2 software. Epoch lengths were set to one minute. Raw data was extracted and analyzed using Action4 software to determine circadian rhythm parameters. Data for each subject were averaged over 21 days and used to determine group comparisons.

Certificate of Confidentiality. A certificate of confidentiality was obtained from the National Institutes of health to protect identifiable information passed from participants to researchers. Certificate allowed researchers to refuse the disclosure of participant identifiers to “any civil, criminal, administrative, legislative, or other proceeding at the federal, state, or local level” (<http://grants.nih.gov/grants/policy/coc>; accessed August 2, 2012, last updated July 23, 2012). A description of the certificate was included in the informed consent.

Procedure

Intake/Initial Visit. Individuals were invited to the Sleep and Actigraphy Laboratory on James Madison University’s main campus. After briefing participants on the goals of the study, all participants signed an informed consent form. During initial assessment, participants filled out an intake form indicating personal marijuana use and previously diagnosed psychiatric disorders. Urine samples were collected from participants who provided both informed and intake consents, regardless of reported use. Urine samples were tested subsequently using the THC strip-test. Results of urine tests indicated group membership, control (1) or marijuana (2). On return to the Sleep and Actigraphy Lab, participants filled out demographic information, prescription medications, and over-the-counter supplements used. After information was collected, participants received actigraphs and sleep/substance use

diaries. Participants were educated on diaries as well as proper handling and use of actigraphs. Before participants exited, a return date for the ANAM was scheduled.

Second Visit. During the second visit to the lab, participants were given the ANAM. Researchers downloaded participants' actigraphs during ANAM administration. After completing the assessment individuals received additional sleep/use diaries if necessary and a final date was established for return of actigraph.

Final Visit. Participants were asked to provide a second urine sample, which was subsequently tested, and results recorded. Actigraphs were downloaded and participants filled out the exit survey. After all materials were collected participants were debriefed about the study and provided the opportunity to receive feedback on their sleeping patterns during their participation.

Results

Participants showed 100% compliancy with actigraphy, all wearing the actigraph for the duration of their participation in the study. Within the control group, 1 participant reported smoking marijuana during participation in the study, and was administered an additional urinalysis. The test returned negative and their data was maintained in the control group. All individuals who reported smoking marijuana greater than 5-7 times a week tested positive on both urinalyses, and individuals who did not report smoking marijuana greater than 5-7 times a week tested negative. On average, the MJ group began chronically smoking at the age of 14 ($SD = 2.04$) and had been chronically using for 6 years ($SD = 2.83$). As a group, the marijuana users engaged in use an average of 106.12 times ($SD = 145.01$) during the 21 days; however, method of administration was not reported. Other drug was reported

throughout study (see Appendix D). No participants reported using illicit drugs other than marijuana. All participants scored as intermediate chronotypes on the MEQ (MJ = 47.18, Control = 47.85), suggesting all participants had similar preferred sleep-wake schedules.

Circadian Rhythms

Regression analyses were conducted to determine whether age when chronic MJ use began and total amount of MJ use during study predicted circadian rhythm alterations in chronic users. These results were non-significant ($F_{change} = 1.279$, $p = .309$). However, marijuana users' mean sleep-wake rhythms ($R^2 = .503$) were significantly better fit by the cosine model than non-users ($R^2 = .435$), $t = -2.075$, $p = .047$, $d = .76$), showing that the MJ group was more entrained to the 24-h model than the non-users (see Appendix C and D for examples of data and cosine model, respectively). Trends for stronger 24-h rhythms, without model assumptions, were also present in the marijuana group ($r = .333$) when compared to control ($r = .276$), $t = -1.796$, $p = .083$. Again, revealing the MJ group as more entrained than non-using controls.

Sleep

Significant differences were found on wake after sleep onset (WASO) between MJ users ($M = 52$ min $SD = 25$ min) and non-users ($M = 45$ min $SD = 45$ $SD = 40$) $U = 16656$ $p < .001$ $r = .23$. Also, a trend for later wake times was found for marijuana users ($M = 8:57$ AM $SD = 1$ h 58 min) when compared with controls ($M = 8:33$ AM $SD = 1$ h 29 min), $p = .061$. No significant differences were found between

MJ users and non-users on TST, (MJ $M = 429.77$ min $SD = 61.54$ min; Control $M = 441.46$ min $SD = 40.50$ min) or sleep onset (MJ $M = 1:02\text{AM}$ $SD = 1.37$; Control $M = 12:48\text{AM}$ $SD = .89$). See Appendix E for all sleep quality parameters.

Neurocognition

Higher reports of marijuana use, in the marijuana group, negatively correlated with percent correct on the CSI (immediate memory task) ($r = -0.593$, $p = .02$) and number of correct responses on the go-no-go task ($r = -0.716$, $p = .001$), with individuals who smoked more receiving lower scores on both tasks. Also, between-group trends were found on the CSI ($M_{MJ} = 960.89$ $SD = 214.11$; $M_{control} = 829.16$ $SD = 88.03$), $p = .064$. However, no significant between group differences on ANAM were revealed.

Discussion

Legal pressures and changing political views around the United States have incited a discussion about marijuana use and as a result, have encouraged new research efforts. Studies are now examining negative, and potential positive effects, of marijuana as well as the biological mechanisms underlying the overall effects cannabinoids have on the human body. This study was a first to examine the relationship between marijuana use and circadian rhythms in a human population and provided some evidence that marijuana use may assist 24-h entrainment but also may induce significant sleep disturbances and slight cognitive impairments.

Data from the current study depicted chronic marijuana users significantly more entrained to the traditional 24-h Cosiner model than age-matched, non-using

controls. Trends for more robust 24-h rhythmicity in marijuana users when compared to controls presented as well as trends toward later wake times for MJ users. No significant between-group differences on TST were found; however, marijuana users did demonstrate increased sleep disturbances when compared to non-users. Also, as predicted, heavier marijuana use throughout the study was associated with increased neurocognitive deficits.

These data are the first to examine circadian alterations in non-treatment seeking, human chronic marijuana users. Previous animal studies (Acuna-Goyocolea et al., 2010; Sanford et al., 2008) suggested circadian alterations, particularly phase advancements. In contrast, the current study demonstrated no phase advancement, but in fact found that the MJ group had later wake times and bedtimes when compared to controls, although it was not significant.

Because participants' drug use was not limited to cannabis, one could assume that other drugs of choice may have been acting to confound the effects of marijuana. However, it is important to note both MJ and control groups engaged in similar amounts of other, non-marijuana drug use suggesting use of other drugs could not completely account for the reversal in the expected between-group relationship. Additionally, marijuana users in the current study had various zeitgebers acting on and affecting their rhythmicity, unlike the relatively cue-deprived animals in discussed experiments. The interaction between chronic use and environmental contingency may act together to affect expression of the rhythm.

Marijuana users were significantly more entrained when compared to controls. Earlier studies exploring circadian rhythms and drug addiction suggest chronic drug use commonly results in severe circadian alterations, not further entrainment (Jones et al., 2003; Wasielewski & Holloway, 2001). However, other studies indicate psychoactive drugs of abuse (cocaine, methamphetamine, fluoxetine) can assist in entraining SCN-independent pacemakers (Kosobud et al., 2009; Manev & Uz, 2006). These SCN-independent pacemakers can be found in the parietal cortex, nucleus accumbens, and caudate putamen, all areas of the brain also affected by cannabinoids (Akhisaroglu et al., 2004; Breivogel & Childers, 1998; Falcon & McClung, 2009; Masubuchi, et al., 2000). No literature detailing whether THC or cannabinoids can induce similar results has been conducted, however, it seems plausible to assume chronic assumption of marijuana may result in similar entrainment outcomes based on current data.

Another explanation surrounds circadian entrainment as a function of drug administration. Individuals in the marijuana group may have experienced an additional zeitgeber in marijuana use, further entraining their circadian rhythms. Hart et al (2005) found that when given a choice, marijuana users exhibited preferential timing for use of marijuana, consistently choosing evening times. This was supported with animal literature confirming there are daily periods of increased motivation to seek and use drugs of abuse (Kosobud et al., 2007; Manev & Uz, 2006). Further, animals when deprived of all other zeitgebers, except drug and its administration, will entrain to daily injections of drugs of abuse (Kosobud et al., 2007). Even more, animals with ablated SCN's can still entrain to drug administration in ways similar to

how animals entrain to food administration (Kosobud et al., 2007). This describes the strength of the SCN-independent oscillators and their ability to produce sustainable rhythms in the absence of SCN input; further implicating not only the SCN but also the SCN-independent pacemakers in the circadian entrainment associated with drug abuse (Kosobud et al., 2007). Again, this effect has not been studied with chronic administration of marijuana. Yet, the literature combines to suggest chronic self-administration of marijuana, if occurring at similar times of day, may impact the SCN-independent oscillators, similar to other drugs of abuse, resulting in increased entrainment.

Limited differences were seen between groups on most sleep parameters and no differences were seen between groups on the ANAM. The narrow difference seen between groups on sleep parameters may be explained by sampling restrictions. College students are known to exhibit extremely poor sleep hygiene and are commonly perceived as a sleep deprived population (Lund et al., 2009). The amount of within-group variability with regard to sleep parameters, specifically in the control sample, was large making it difficult to weight any differences seen between-groups. Increasing sample size may account for some of the within variance and may allow for clearer between group comparisons. Also, expanding the population from college students to a more representative sample of community members could potentially account for the high amounts of variability. College students are at a vulnerable developmental stage with regard to sleep regulation (Dahl & Lewin, 2002). It has been shown that the adolescent brain undergoes a series of maturation changes around the age of 21, similar to the mean age of participants in the current study. These

developmental changes have a significant impact on sleep and circadian rhythms; particularly sleep onset and TST (Dahl & Lewin, 2002). However, many of these changes are subject to individual differences and in no simple way controlled for in an experimental design. Before generalization of the current study's results, it would be important to look at the same phenomenon in a sample not undergoing these biological changes.

Data are very controversial with respect to cognitive deficits and marijuana use. Some researchers advocate marijuana use is associated with a range of long-lasting cognitive deficits (Meier, 2012), while others reject the notion of significant cognitive deficits as a result of marijuana use (Iverson, 2005; Pope et al., 2001; 2002). Our results indicate that while immediate memory processing and appropriate inhibitory control may decrease with increased use, the decrease in these cognitive functions is not significant when compared to non-marijuana users. So, although heavier marijuana users may experience more cognitive deficits based on our data, those deficits do not seem to significantly impact their overall cognitive abilities when compared to non-using peers.

The current study's focus on non-treatment seeking individuals who did not have previous substance abuse diagnoses was a considerable strength. Considering, that nine percent of individuals who use marijuana will become dependent, the much greater majority of individuals will not (NIDA, 2012). Although it is very important to continue to develop interventions and treatments for dependence, this study focused on and attempted to understand marijuana use outside the extreme of dependence. Further, we utilized actigraphy to monitor individuals in their natural,

home environments, without the constraints of a laboratory setting. Many previous studies monitoring marijuana users have focused on self-report sleep data or monitored users over one or two nights in a sleep lab. The design of the current study allowed for extensive, objective monitoring of individuals' sleep and circadian patterns over a period of three weeks; eliminating the possibility of first-night effects or unrepresentative results. These strengths assist in overall generalizability of the study, but also provide significant limitations with regard to experimental control.

We recruited non-treatment seeking users and monitored naturally occurring behavior patterns and as a result, we did not possess the same degree of control many studies that engage treatment facilities own. Consequently, we relied solely on self-report measures for drug intake and total use and could not accurately define overall use throughout the study. Further, we could not control for time of use each day. Retrospectively, we would hope to have increased control over the time of day cannabis was ingested and have better report measures for method of administration.

Future Directions

These data are the first to examine the relationship between chronic marijuana use and human circadian entrainment, and while results are suggestive, much more research needs to follow to help further examine the phenomenon. The above results should be replicated in a non-collegiate sample to assist with better generalizability of results. Further, chronic adult users should also be examined and circadian-related developmental differences between college users and adults explored. Also, future iterations should control the time of day marijuana use occurs to explore the

immediate effects of time misperception and marijuana intoxication. Additionally, control over time of day of use will help tease apart confounding factors that could be contributing to entrainment.

A connection between marijuana dependence and sleep has recently been made and previous studies have connected other substance addictions to sleep and circadian rhythms. This study is the first to suggest circadian rhythms as a driving mechanism connecting sleep and chronic marijuana use, but the many unanswered questions remaining highlight the importance of further investigation into this important topic.

Appendix A

Participant recruitment email

“Dear Potential Participant,

You are invited to a study conducted by the James Madison University Sleep and Actigraphy Lab. This study intends to examine the effect of marijuana on biological and cognitive processes. Because of the sensitive nature of the topic all information provided to researchers is protected and confidential. All participants have the opportunity to WIN up to \$150 in a blind raffle at the conclusion of the study.”

Appendix B

Sleep Diary

Participant Number _____

To be filled out just before you go to bed:

Date: _____

1. Did you take any naps? _____

1a. If yes: When? _____ How long? _____

2. How many cups of caffeinated beverages did you consume and what were they?

3. What time of day did you have the caffeinated beverages?

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

5. Did you take the actigraph off for any period during the day? If so when and for how long?

6. Did you consume/smoke marijuana today? How many hits/blunts/sessions?

7. Did you consume any other drug substances today? What were they? (Exclude prescription medications)

To be filled out upon waking up in the Morning:

Date: _____

1. What time did you first try to go to sleep last night? _____

2. How long did it take you to fall asleep last night? _____

3. How many times did you wake up during the night? _____

3a. How many total minutes were you awake last night after you first fell asleep?

4. What time did you wake up this morning? _____

5. How many total hours of sleep did you get last night? _____

6. Are you sleepy this morning? YES NO

Appendix C**Table 1.**

Demographics

	<i>N</i>	<i>Age</i>	<i>Gender</i>	<i>Enrolled JMU Students</i>
<i>MJ</i>	17	21.47	9 (F)	16
<i>Control</i>	13	21.54	8 (F)	12
<i>Total</i>	30	-	17(F)	28

Appendix D**Table 2.**

Participant drug use by group

	<i>Alcohol</i>	<i>Nicotine</i>	<i>Caffeine</i>	<i>OTC</i>
<i>MJ</i>	13	1	7	5
<i>Control</i>	6	1	4	6

Note: Numbers indicate number of individuals in each group engaging in use of substance

Appendix E**Table 3.**

Mean sleep parameters

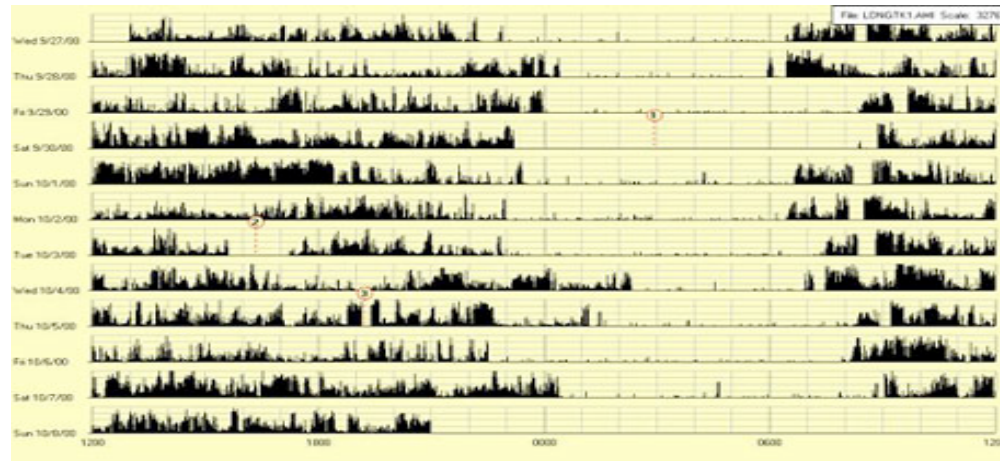
	<i>Sleep Onset</i>	<i>Wake Time</i>	<i>TST</i>	<i>WASO*</i>
<i>Mj</i>	1:02AM	8:57AM	7 h 20m	52m
<i>Control</i>	12:48AM	8:33AM	7 h 50m	45m

Note: (indicates statistical significance)*

Appendix F

Figure 1.

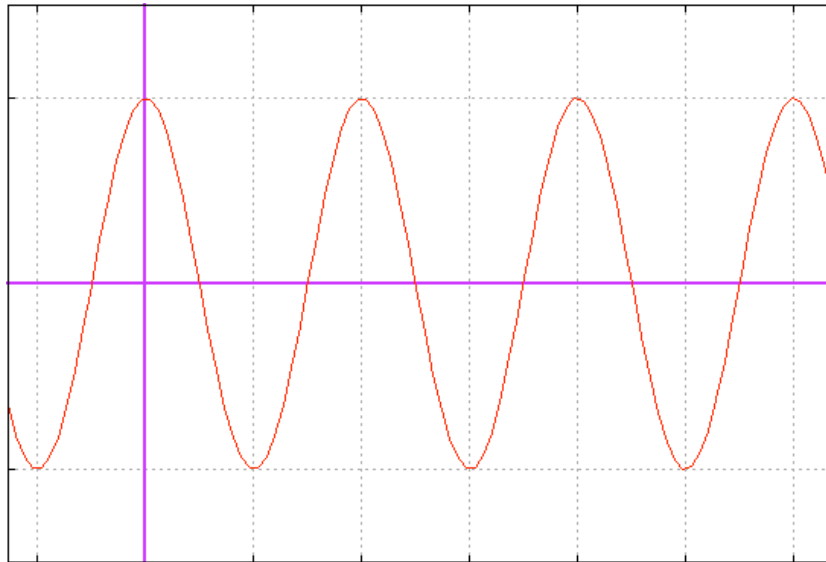
Sample actigraphy data



Note: Data output fit to cosine model (Figure 2) to establish model fit and 24-h entrainment

Appendix G**Figure 2.**

Sample cosine wave



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