The Effects of Oral Nicotine Administration on Sleep Quantity and Architecture in Female C57BL/6J Mice

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Abstract

Background: Nicotine dependence is often associated with sleep disturbances. The consequent daytime sleepiness of poor sleep may encourage the user to continue nicotine use, for the drug exerts an acute stimulative effect. Researching the mechanistic effects of nicotine on sleep will provide useful information for the pharmacological treatments of addiction. The female sex has been excluded from research in this area thus far. The objective of this study was to investigate the effects of oral nicotine administration on sleep in female C57BL/6J mice.

Methods: N=4 female C57BL/6J mice were given *ad libitum* access to a 200 µg/mL nicotine solution. Electroencephalogram and electromyogram activity was recorded continuously for two weeks.

Results: Nicotine exerted three main effects on sleep: an increase in non-REM sleep, a decrease in REM sleep, and a decrease in stage-shift transitions. These effects were only significant during the inactive phase.

Conclusions: These results provide evidence for the mechanistic action of nicotine on sleep in female mice. These effects together may be a result of receptor desensitization of nicotinic acetylcholine receptors, which are involved in the neuronal pathways that transition the brain from non-REM sleep to wakefulness or non-REM to REM sleep. Further research should aim to confirm dose-dependent receptor desensitization of relevant brain regions. Additional spectral analyses of the gathered EEG data should be performed.

Introduction

Nicotine addiction remains a pervasive health problem. The detrimental consequences of smoking have been well known for decades, yet nicotine dependence persists. Nicotine is carcinogenic, immunosuppressive, and correlated with a myriad of undesirable health conditions (Mishra et al., 2015). Despite public health initiatives and governmental control of cigarettes (e.g., age restrictions, targeted taxation, product warning labels, and public area smoking bans), 15.6% of adult men and 12.0% of adult women currently smoke in the United States (World Health Organization, 2016; Center for Disease Control, 2018). Many smokers express a desire to quit, but the endeavor is a difficult one.

Nicotine addiction is a cyclical mechanism. The user may wish to quit, but the unpleasant side effects of its withdrawal are commonly alleviated with the reintroduction of nicotine. A frequent side effect of nicotine withdrawal is sleep disturbance. Nicotine may affect a person's sleep quality or sleep quantity through several proposed mechanisms, among them: nicotine-induced release of neurotransmitters that affect the sleep cycle; acute withdrawal while sleeping (and therefore abstaining from smoking); or secondary causes of other disorders (i.e., pulmonary disorders) (McNamara et al., 2014). Nicotine users report decreased total sleep time and increased sleep-onset latency (Jaehne et al., 2009). These side effects cause daytime sleepiness (Jaehne et al., 2009), which may encourage users to consume stimulative nicotine.

While these subjective accounts give evidence of nicotine's effect on sleep, there presently exists an inconsistency within objective data on the subject. Studying sleep architecture (the quantitative composition and timing of sleep stages and cycling) through polysomnography (PSG) provides useful data for the mechanism of action that nicotine has on sleep. This

information may identify specific sleep aids as more useful than others to ameliorate the effects of inefficient or insufficient sleep consequential of the altered neurochemistry.

Mice have become popular subjects in the field of sleep science, as the cost and logistics of human subjects necessitate a shorter study duration. One question that arises is the transferability of information gained from mouse brains to human brains. However, mice share similar brain architecture, neurochemicals, and sleep-stage cycling to humans (Paterson et al., 2011), and are therefore accepted as suitable subjects.

In particular, female mice have not been studied in this area. It is an objective of the decade to include the female sex more broadly in medical research. As of 2016, the National Institutes of Health (NIH) require funding applicants to detail how their research will include the biological variable of sex in their design and analyses (NIH, 2016). The purpose of this study was to assess the effects of nicotine administration on C57BL/6J female mice. The inclusion of the female sex in this research may expose different effects of nicotine on sleep, which has implications for the pharmacology in female humans.

Male C57BL/6J mice have demonstrated reduced total sleep time and NREM sleep under similar conditions (Mathews & Stitzel, 2018). Due to the acute stimulative effects of nicotine, we expected that female mice would experience decreased total sleep time, decreased NREM sleep, and increased REM sleep.

Methods

Design and Procedure

This study was performed at the Institute for Behavior Genetics (IBG) in Boulder, Colorado. The study was approved by the Institutional Animal Care and Use Committee

(IACUC) to ensure animal wellbeing. Nine-week-old C57BL/6J female mice were chosen as subjects, n = 4. This strain of mouse has demonstrated that its females' sleep is not majorly regulated by the estrous cycle, nor does the estrous cycle affect sleep (Koehl et al. 2003).

The mice underwent surgery, anesthetized under isoflurane, to install a cortical head mount (Pinnacle Technology, Inc., Lawrence, KS) using two stainless steel screws at the location supplied by Pinnacle Technology, Inc. The screws served as electrodes for EEG data acquisition. Two stainless steel intramuscular EMG electrodes were placed on the mice's nuchal muscles on the dorsal neck. Muscle activity can aid differentiation between sleep stages, as mice experience muscle atonia during REM and muscle movement during wakefulness. Following surgery, the mice were given a 0.1mg/kg subcutaneous injection of slow-release Buprenorphine for pain, then acclimated in their housing units for 7 days post-surgery. The housing units (Pinnacle Technology, Inc., Lawrence, KS) contained standard nesting materials: cotton pads and shredded paper. The units were kept in a quiet room in the IBG Vivarium. Lights were kept on a light/dark routine, turning on at 6 am and turning off at 6 pm.

Following the seven-day recovery period, the head mounts were then attached to computer cables, which were fed through a hole in the roof of the housing unit. The mice adjusted to the cable attachment for one week. After adjustment to the head mount and cable attachment, recording of EEG and EMG data began. The diagram below demonstrates the experiment timeline (Figure 1).

The nicotine was administered *ad libitum* at a concentration of 200 µg/mL, within a vehicle of water and 0.2% saccharin. Orally administered nicotine is efficacious in delivery, showing comparable plasma levels to other administration routes (Matta et al., 2007). The drinking solution was replaced every 3-4 days throughout the experiment. Mice were weighed

before the EEG-electrode surgery and after the experiment. Nicotine consumption was tracked volumetrically through the consumption of the drinking solution.

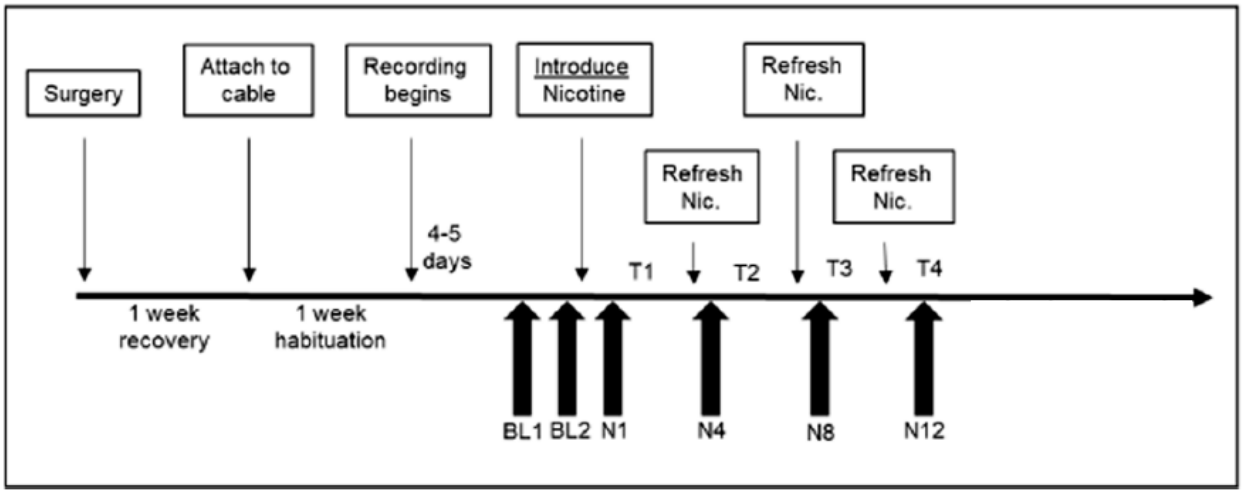

Figure 1. Timeline of experiment scheme. Top arrows indicate experimental procedures. Bottom arrows indicate days for which EEG and EMG recordings were scored. Period, T, represents the period between experiment procedures. Figure adapted from "The Effects of Oral Nicotine Administration and Abstinence on Sleep in Male C57BL/6J Mice" by Mathews & Stitzel, 2018.

Data Acquisition and Analysis

EEG and EMG activity was recorded using the Sirenia Acquisition program (Pinnacle Technology Inc., Lawrence, KS) at a sampling frequency of 400 Hz. This data was collected continuously for the 3-week experiment. Data were condensed into 24-hour periods. The last two days of baseline were averaged. Days 1, 4, 8, and 12 of nicotine administration were also averaged after one-way ANOVA tests indicated no significance between days for all variables ($p > .05$).

The sleep scoring program used was Sirenia Sleep Pro 1.7.4 (Pinnacle Technology Inc., Lawrence, KS). Scoring epochs were set to 4 seconds. The EMG and EEG data were used collectively to identify and score sleep stages using these characteristics: high activity EMG and high frequency, low amplitude EEG data were scored as wakefulness; low activity EMG and low frequency, high amplitude EEG data indicated NREM; and low activity EMG and high

frequency, low amplitude EEG data indicated REM. Scoring was first performed through an automated function in the program, then manually reviewed.

The data were compared within three timeframes: the 12-hour lights-on period (inactive phase), the 12-hour lights-off period (active phase), and the entire 24-hour period. Sleep quantity was assessed through these variables: total sleep time and percentage of time in NREM and REM. Sleep architecture was evaluated by the duration and frequency of sleep stage bouts, total stage shifts (transitions between one stage to another), and sleep stage shifts (transitions from sleep to wake or vice versa). A bout is defined as four seconds or longer spent continuously in one stage. The data were analyzed using the statistical and graphing program GraphPad Prism 9.3.1 (GraphPad Software Inc., San Diego, CA). Paired two-tail t-tests were used between the baseline and nicotine treatments, alpha = .05.

Results

Nicotine Consumption Across the Experiment

Nicotine consumption was measured four times during the experiment. The average daily consumption was calculated and standardized by animal weight. Animals were weighed before EEG-electrode surgery and after the experiment. Average weight before surgery was 20.15 (± 0.56) grams and average weight after the experiment was 19.75 (± 0.74) grams.

Consumption during the first measurement period, T1, was 88.92 (± 5.08) mg/kg/day (Figure 2). Consumption during the subsequent measurement periods was less. The consumption during T2, T3, and T4 were 61.95 (± 2.07), 72.15 (± 4.89), and 68.38 (± 1.87) mg/kg/day, respectively (Figure 2.A.). The average nicotine consumption across the experiment was 72.85 (± 2.63) mg/kg/day. The average daily fluid intake was 7.25 (± 0.18) mL/day.

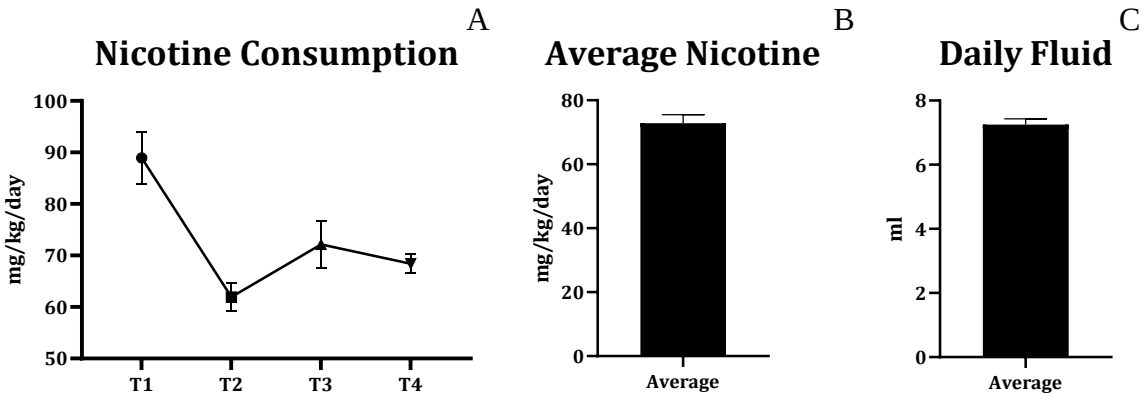

Figure 2. ***A.*** *Average consumption of 200 µg/mL of nicotine in a .02% saccharin solution. Period, T, represents the period between solution volume measurements. Data expressed as average ± standard error of the mean (n = 4).* ***B.*** *Average consumption of 200 µg/mL of nicotine in a .02% saccharin solution for the experiment's duration. Data expressed as average ± standard error of the mean (n = 4).* ***C.*** *Average daily consumption of fluid for the experiment's duration. Data expressed as average ± standard error of the mean (n = 4).*

Effect of Nicotine Administration Within a 24-Hour Period

The EEG and EMG data were used to measure the effects of nicotine on sleep architecture and sleep quantity. A one-way ANOVA test indicated no significance between days 1, 4, 8, and 12 of nicotine, therefore the data for these variables were averaged. Several tests indicated significance within the 24-hour period. Total sleep time, expressed as a percentage of the 24-hour period, was not significantly different across treatments. However, categorizing the periods during which the animal was sleeping within the 24-hour period does provide two insights. Nicotine administration led to an increase in the percentage of time during sleep spent in NREM from pre-nicotine baseline ($t(3) = 5.008$, $p = .0153$) (Figure 3). The percentage of time during sleep spent in REM decreased from baseline ($t(3) = 5.006$, $p = .0153$) (Figure 3).

Sleep architecture can also be examined as percentages of the 24-hour period. The percentage of time in NREM during nicotine administration increased from baseline, but this result did not reach significance ($t(3) = 2.913$, $p = .0618$) (Figure 4) (Table 1). The frequency of NREM bouts across treatments was not signifhicant, but the duration of NREM bouts increased

during nicotine administration (t (3) = 3.819, p = .0316) (Figure 5). The percentage of time in REM decreased from baseline (t (3) = 4.023, p = .0276) (Figure 4) (Table 1). The frequency of REM bouts during nicotine administration also decreased from baseline (t (3) = 3.220, p = .0486) (Figure 6). There was no significant difference in REM bout duration across treatments. Total stage shifts over the 24-hour period decreased from baseline (t (3) = 3.781, p = .0324) (Figure 7). Sleep stage shifts also decreased from baseline (t (3) = 3.416, p = .0420) (Figure 7).

In summary, total sleep time was not significantly affected by nicotine administration. NREM sleep increased as a result of the increased bout duration. REM sleep decreased as a result of the decreased bout frequency. Fewer total and sleep stage shifts occurred during nicotine administration than during baseline.

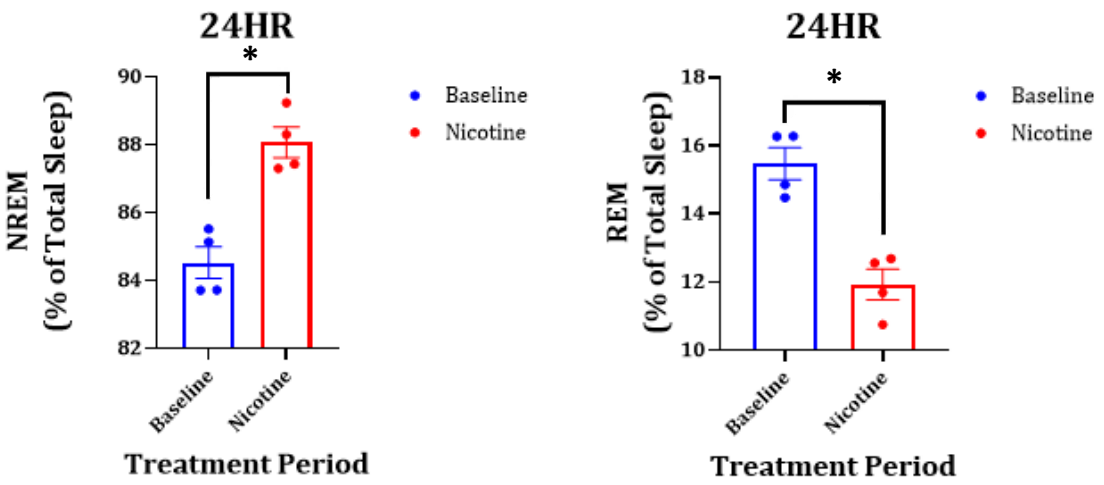

*Figure 3. Average percentage of time in NREM and REM during sleep. Data expressed as average ± standard deviation of the mean (n = 4). *p < .05, alpha = .05.*

Effect of Nicotine Administration During Active and Inactive Phases

The 24-hour period was further analyzed under two 12-hours periods: the lights-on, inactive phase and the lights-off, active phase. No significant difference was observed across treatments during the active phase, nor did any results approach significance. During the inactive

phase, several effects were observed. The percentage of time in NREM sleep upon nicotine administration increased from baseline ($t(3) = 3.364$, $p = .0436$) (Figure 4) (Table 1). The duration of NREM bouts ($t(3) = 5.354$, $p = .0127$) increased from baseline, but the bout frequency decreased ($t(3) = 3.326$, $p = .0449$) (Figure 5). REM sleep as a percentage of the inactive phase decreased from baseline ($t(3) = 6.147$, $p = .0087$) (Figure 4) (Table 1). The frequency of REM bouts decreased upon nicotine administration ($t(3) = 4.421$, $p = .0215$) (Figure 6). The duration of REM bouts was not significantly different across treatments. The amount of total stage shifts across the inactive phase decreased from baseline ($t(3) = 4.298$, $p = .0232$), as did the amount of sleep stage shifts ($t(3) = 3.198$, $p = .0494$) (Figure 7).

In summary, no effect was seen during the active phase during nicotine administration. During the inactive phase, NREM sleep increased as a result of increased bout duration. NREM bout frequency did decrease from baseline, but this effect was outweighed by the increased bout duration. REM sleep decreased as a result of decreased REM bout frequency. Nicotine administration had the effect of fewer total and sleep stage shifts.

*Table 1. Total sleep time, NREM, and REM expressed as average percentages ± standard deviation of the mean (n = 4). Significance is compared to baseline. * p < .05, ** p < 0.01, alpha = .05.*

	24 Hour		Active		Inactive	
	Baseline	Nicotine	Baseline	Nicotine	Baseline	Nicotine
Sleep	37.59 ± 2.846	38.71 ± 1.878	17.49 ± 6.259	17.27 ± 4.880	57.70 ± 1.000	60.16 ± 3.189
NREM	31.81 ± 2.720	34.11 ± 1.523	15.54 ± 5.706	15.51 ± 4.161	48.06 ± 0.9640	*****52.71 ± 2.898
REM	5.795 ± 0.1709	*****4.605 ± 0.4786	1.955 ± 0.5664	1.762 ± 0.7183	9.636 ± 0.3378	******7.449 ± 0.6680

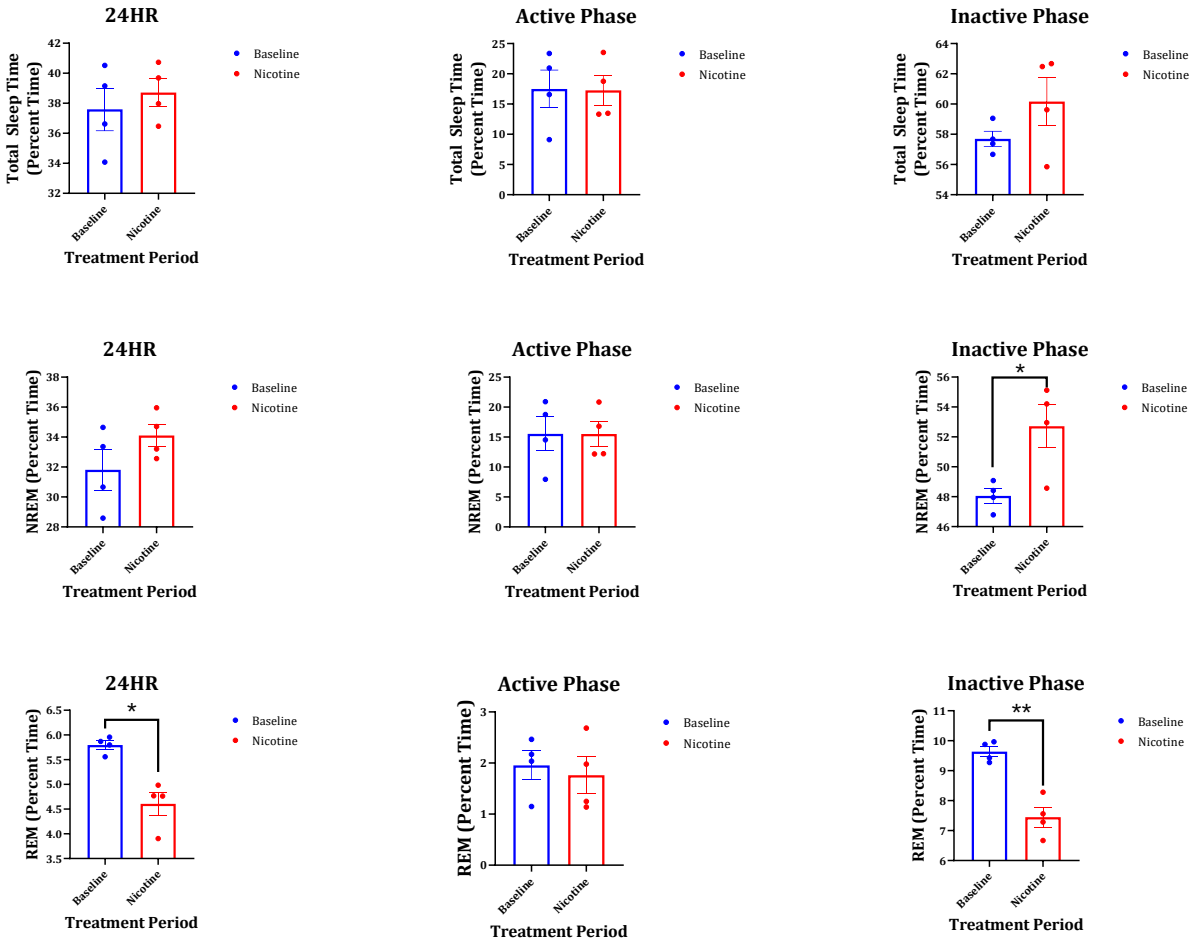

*Figure 4. Total sleep time, NREM, and REM percentages of 24 hours and separated into the 12-hour active and inactive phases. Significance is compared to baseline. Data expressed as average ± standard deviation of the mean (n = 4). * p < .05, ** p < .01, alpha = .05.*

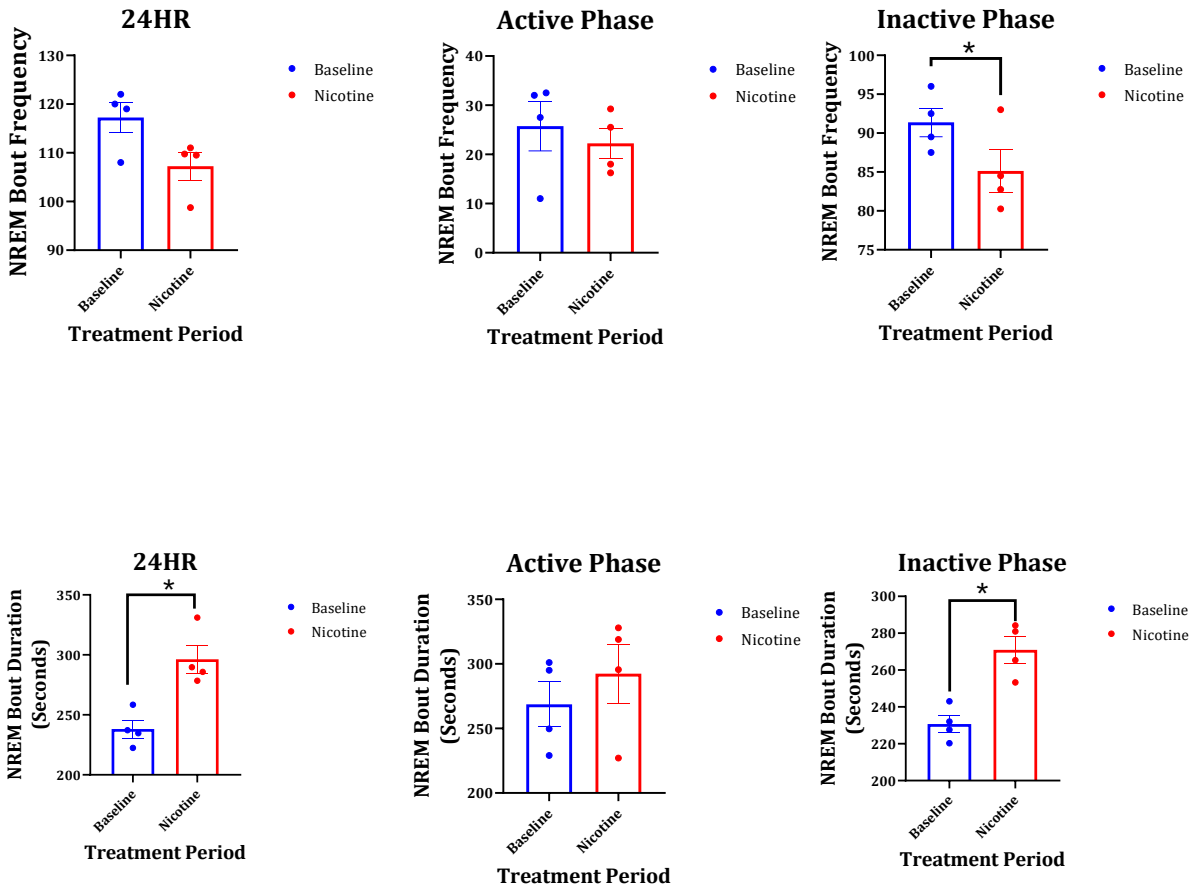

*Figure 5. Average bout frequency and duration of NREM sleep for 24 hours and separated into the 12-hour active and inactive phases. Data expressed as average ± standard deviation of the mean (n = 4). *p < .05, alpha = .05.*

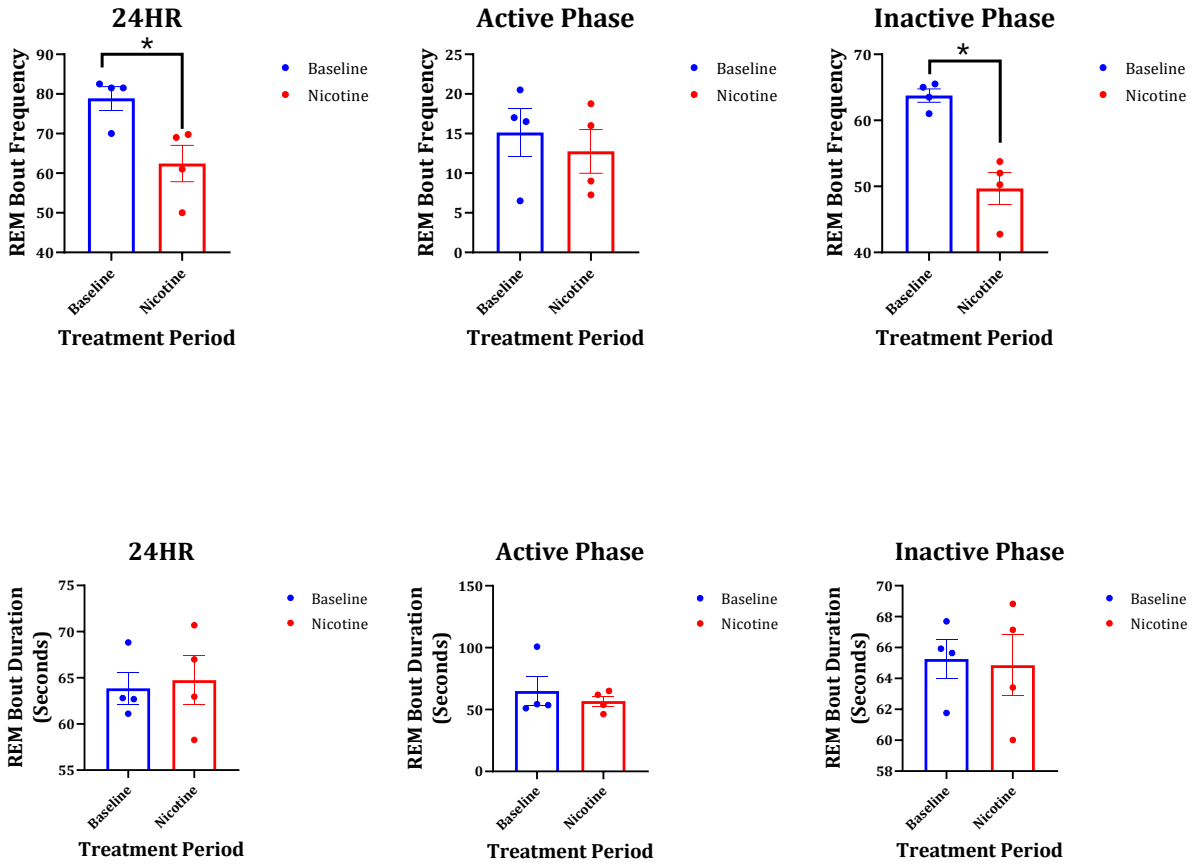

*Figure 6. Average bout frequency and duration of REM sleep for 24 hours and separated into the 12-hour active and inactive phases. Data expressed as average ± standard deviation of the mean (n = 4). *p < .05, alpha = .05.*

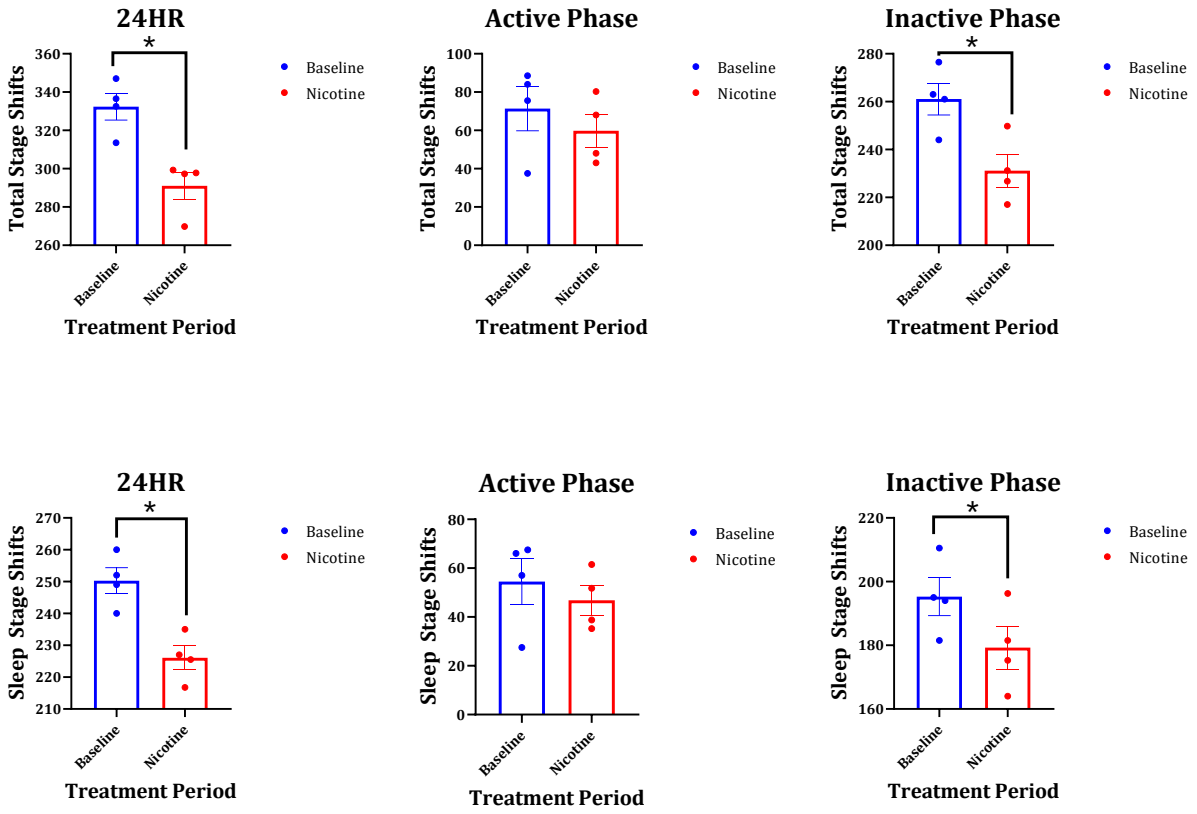

*Figure 7. Average number of total stage shifts and sleep stage shifts for 24 hours and separated into the 12-hour active and inactive phases. Data expressed as average ± standard deviation of the mean (n = 4). *p < .05, alpha = .05.*

Discussion

Three general observations were drawn from this study: nicotine increased the amount of NREM sleep, decreased the amount of REM sleep, and decreased the number of total stage shift transitions. These results may be a consequence of nicotine inhibiting the transition from NREM into REM. This explanation would account for the decrease in total stage shifts. Additionally, impeding the transition into REM sleep would increase the duration of NREM bouts, an effect observed in this study. An additional effect nicotine had on sleep was the decreased number of sleep stage shifts. These are defined as transitions between wakefulness and sleep, or between sleep and wakefulness. While nicotine did not significantly increase TST, there was an average

increase between conditions during the 24-hour period and the inactive phase. These results suggest that nicotine may have inhibited the transition out of sleep. These inhibited transitions into wakefulness and REM may be induced by desensitization of the nicotinic acetylcholine receptor.

Nicotine and the Nicotinic Acetylcholine Receptor

The nicotinic acetylcholine receptor (nAChR) is a pentameric receptor found on the presynaptic and postsynaptic membrane of neurons. Several subtypes of the receptor are known, including a homeric receptor comprised of α7 subunits, and a heteromeric receptor comprised of a mixture of 2 α subunits (α2 - α6) and 3 β subunits (β2 - β4). Nicotine acts as an agonist for the nAChR, with the highest affinity for receptors containing α4 or β2 subunits (Brunzell, 2008).

nAChRs are susceptible to desensitization under chronically elevated concentrations of its ligands. Desensitization is a phenomenon wherein a receptor may be bound to its ligand, but it does not produce a response. In β2-containing nAChRs, this is an intrinsic process that occurs within seconds of receptor activation (Picciotto et al., 2007). Further exposure to ligand during the desensitized state may alter the nAChR conformation again, into an inactivated state. Throughout the nervous system, nAChRs exist in the different states (activatable, desensitized, and inactivated) and are reversibly transitioning between them, though the kinetics of transitioning is dependent on ligand concentration (Brunzell, 2008).

Receptor upregulation typically follows desensitization/inactivation, a process thought of as a compensatory measure of receptor replacement (Dani & Heinemann, 1996). The mechanism through which upregulation occurs is still being researched, but proposed is a second-messenger cascade initiating the transport of stored nAChRs from the endoplasmic reticulum to the

membrane surface (Darsow et al., 2005). Sparks and Pauly (1998) demonstrated that orally administered nicotine (200 μg/mL in a 2% saccharin solution) was sufficient to induce α4β2 nAChR upregulation in female C57BL/6J mice, justifying this study's belief that receptor desensitization occurred during the experiment. Chronic nicotine's effect on receptors may have relevant consequences for the entry out of NREM sleep or into REM sleep, as acetylcholine and nAChRs are involved in associated neural pathways.

The Role of Acetylcholine in Sleep and Wakefulness

Transitioning between sleep and wakefulness requires a complex network of neurochemical pathways. The term "switch" is often employed to describe the transition between brain states, as the change occurs rapidly and completely, much like that of an electrical circuit switch. Recent research has identified likely candidates for the neural centers of initiation into NREM and REM. Sufficient activation of these centers moves the brain into the center's corresponding state. The activity of these centers is modulated by neurons from other brain regions. It is this modulation that determines which side of the "switch" the brain is on, for the side with the greater activity predominates.

Cholinergic neurons have regulatory effects over the brain centers thought to be involved in the promotion of NREM sleep and REM sleep. These processes are described in the following sections.

The Wake / NREM Switch

The wakefulness switch describes the pathways that transition the brain from wakefulness into NREM, or NREM into wakefulness (Figure 8). The ventrolateral preoptic

nucleus (VLPO) and the median preoptic nucleus (MnPO) together are thought of as the NREM-On switch, as they both promote the initiation of NREM and inhibit wakefulness. During wakefulness, the VLPO and MnPO are necessarily silenced to maintain arousal (Brown et al., 2012; Saper et al., 2010).

Two major regions implicated in the modulation of this switch are the cholinergic neurons of the laterodorsal tegmentum (LDT) and the pedunculopontine tegmental nucleus (PPT), found between the midbrain and the pons. These neurons project cholinergic input to the thalamus, basal forebrain, promoting wakefulness. Additionally, the LDT/PPT project acetylcholine onto the VLPO/MnPO. Activation of nAChRs on the VLPO/MnPO suppresses their activity and inhibits the likelihood of entering NREM (Brown et al., 2012; Saper et al., 2010).

If these nAChRs are subject to chronic nicotine administration and subsequently desensitized, the VLPO/MNPO are less inhibited, and NREM sleep can occur to a greater extent. This was a result seen in our study, as nicotine increased the percentage of time in NREM sleep. Nicotine also decreased the number of sleep-stage shifts, possibly due to fewer transitions out of NREM sleep. During NREM sleep, the VLPO/MnPO release GABA onto the LDT and PPT to inhibit wakefulness (Brown et al., 2012). Transitioning from NREM to wakefulness becomes more likely if acetylcholine inputs to the VLPO/MnPO increase, or if GABA inputs to the LDT/PPT decrease. The transition from NREM to wakefulness may be less likely to occur if nAChR desensitization is present, as NREM initiation is promoted but the transition from NREM into wakefulness is not as directly affected.

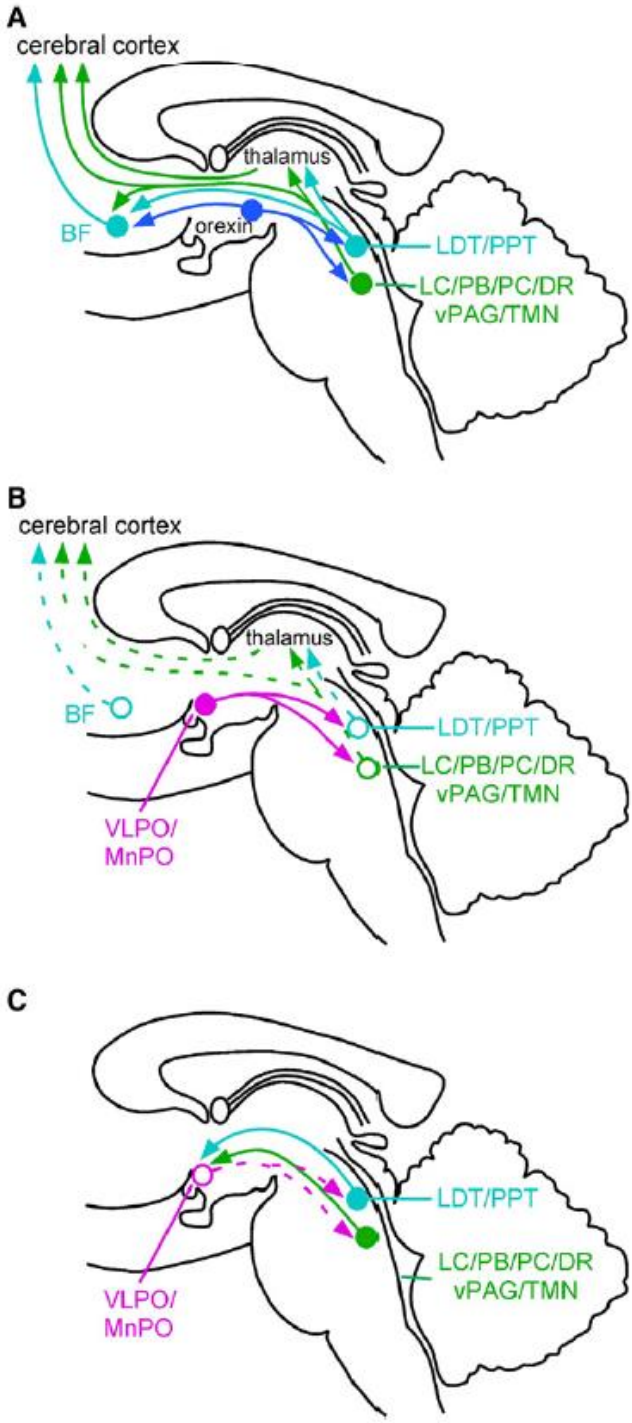

Figure 8. Schematic representation of the Wakefulness/NREM switch. ***A.*** *The laterodorsal tegmentum (LDT) and the peduncular pontine tegmentum (PPT)(aqua) are wakefulness-promoting areas that project acetylcholine onto the thalamus and basal forebrain.* ***B.*** *During NREM sleep, the ventrolateral preoptic nucleus (VLPO) and median preoptic nucleus (pink) are active, and project GABA onto the LDT and PPT. This inhibits wakefulness and promotes NREM.* ***C.*** *During wakefulness, the LDT and PPT inhibit the VLPO from becoming activated. The effect of acetylcholine on the VLPO is a hyperpolarization. Figure reprinted from "Sleep State Switching" by Saper et al., 2010.*

The REM On / REM Off Switch

The REM switch describes the pathways that transition the brain from NREM into REM (Figure 9). The neurons that promote NREM sleep are inhibiting those that promote REM sleep; after the transition into REM sleep, the NREM-sleep-promoting neurons are inhibited.
Two groups of neurons are identified: the “REM-Off cells” and the “REM-On cells”. The REM-Off cells are GABAergic cells within the ventrolateral periaqueductal gray (vlPAG) and the lateral pontine tegmentum (LPT). These neurons project onto the REM-On cells, inhibiting REM sleep from being initiated. The REM-On cells are a group of neurons within the brainstem in the sublaterodorsal nucleus (SLD) and the precoeruleus (PC). These neurons excite the cortex, thalamus, and basal forebrain to promote REM sleep, excite medullary interneurons that project onto skeletal muscle to prevent movement, and inhibit the REM-Off cell group to maintain the state of REM (Brown et al., 2012; Saper et al., 2010).

The LDT and PPT have been shown to also modulate REM sleep. These neurons project acetylcholine onto REM-On cells, making the depolarization of these neurons more likely (Brown et al., 2012; Saper et al., 2010). However, if the nAChRs on the REM-On cells are desensitized, they are no longer responsive to acetylcholine. Therefore, the REM-On cells are not depolarized as often, and REM sleep does not occur as frequently. Additionally, there are REM-Off cells in the LPT that become inhibited by acetylcholine (Brown et al., 2012). If the nAChRs on the LPT are desensitized, there is less inhibition of the LPT, and REM sleep is inhibited. These mechanisms may explain the effect of decreased REM sleep after nicotine treatment. The decreased frequency of REM and the increased duration of NREM may indicate an abnormality in the transition from NREM to REM.

Moreover, optogenetic stimulation of the LDT and PPT has successfully initiated REM sleep bouts but has not been proven to maintain or increase the duration of REM bouts (Van Dort et al., 2014). This is consistent with the results of the present study, as nicotine decreased REM bout frequency, but did not decrease REM bout duration. This effect may be due to the proposed idea that different pathways modulate REM initiation and maintenance (Van Dort et al., 2014).

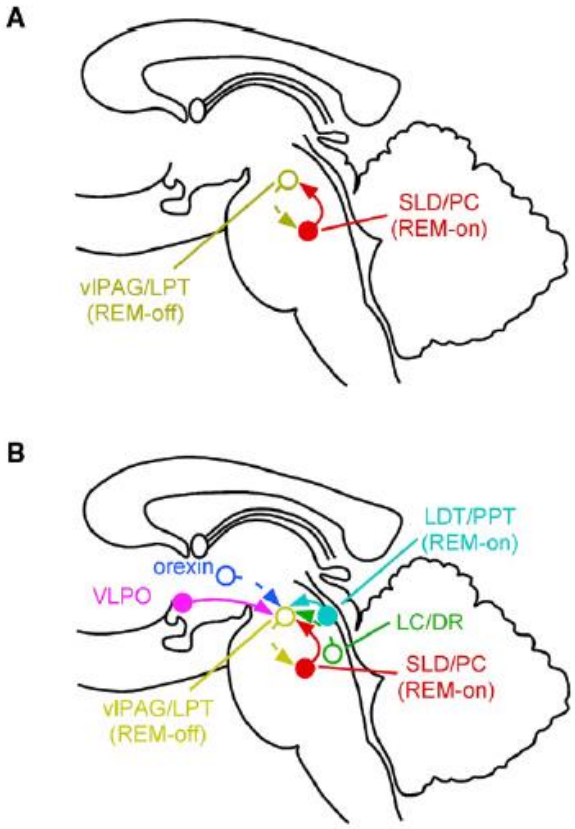

Figure 9. Schematic representation of the REM On / REM Off switch. ***A.*** *During REM, the sublaterodorsal tegmentum (SLD) and the precoeruleus (PC) (red) are actively projecting acetylcholine. Their activity inhibits the effects of the REM-Off area, the ventrolateral periaqueductal gray (vlPAG) and lateral pontine tegmentum (LPT) (yellow).* ***B.*** *Several modulatory inputs to the SLD/PC and vlPAG/LPT are diagrammed. In aqua, the laterodorsal tegmentum (LDT) and the peduncular pontine tegmentum (PPT) promote REM by projecting acetylcholine onto the vlPAG/LPT, inhibiting the REM-Off region. The LDT/PPT also projects acetylcholine onto the SLD/PC, exciting the REM-On region. Figure reprinted from "Sleep State Switching" by Saper et al., 2010.*

Results Compared to Previously Published Studies

The results from this study suggest that orally administered nicotine in female C57BL/6J mice decreases REM sleep, increases NREM sleep, decreases total stage shifts, and decreases sleep stage shifts. These results may be due to nicotine-induced receptor desensitization that prevents the transition from NREM into wakefulness or NREM into REM. This study is partially consistent with relevant literature. Importantly, study results are largely dependent on pharmacokinetics. The effects of nicotine are dependent on drug concentration and timing, for smaller doses often do not induce receptor desensitization. Therefore, the effects of a small dosage may have opposite or opposing effects of a larger dosage. When comparing the results of this study to another, this is an essential consideration.

A previous publication by this laboratory studied male C57BL/6J mice under the same methodology and observed that nicotine decreased TST and NREM sleep (Mathews & Stitzel, 2018). In the present study, TST was not affected and NREM increased. It is worth noting that the male mice consumed an average of 68.2 mg/kg/day of nicotine, while the present female mice consumed an average of 72.85 mg/kg/day. The contradiction in results among sexes may be due to the increased nicotine concentration in females.

The present study is unique in that nicotine is administered chronically and PSG is recorded continuously. In other animal studies, the route of administration is often a daily injection given just prior to EEG recordings. Regardless, other studies have observed similar effects on REM sleep. Salin-Pascual et al., (1999) addressed the dose-dependence of nicotine's effects in a study using rats and intraperitoneal nicotine injections. It was observed that nicotine decreased REM sleep more significantly as the dosage increased. A significant decrease in REM percentage was only achieved at 0.5 mg/kg and 1 mg/kg, but significantly decreased REM

frequencies were achieved at 0.1 mg/kg, 0.25 mg/kg, 0.5 mg/kg, and 1 mg/kg. Jewett and Norton (1966) found a dose-dependent relationship between subcutaneously administered nicotine and REM sleep in cats. In their study, REM sleep increased at 0.05 mg/kg and 0.1 mg/kg. At a concentration of 0.2 mg/kg, REM sleep decreased and TST decreased. Similar results were observed in 1996 in a paper by Vasquez et al. In a study using cats, REM bout frequency was shown to decrease in a dose-dependent manner after nicotine administration.

In human subjects, current cigarette smokers were observed to have a decrease in TST, a decrease in NREM sleep, and an increase in REM sleep as compared to never-smokers (Zhang, 2006). It has also been noted that smokers have a decreased percentage of δ-bandwidth in EEG recordings, implying a reduction in restorative NREM sleep (Zhang et al., 2008). These results are more consistent with the stimulative effects of nicotine and agonistic effects of nAChRs on REM sleep. They contradict the present study, as our study suggested nicotine slightly (though insignificantly) increased average TST, due in part to the decrease in sleep-stage transitions and increased NREM percentage.

Importantly, the Zhang et al. (2006) paper did not track nicotine consumption, categorizing subjects as “never smokers” or “current smokers” by each subject’s report. The 2008 Zhang paper did have a pre-requisite qualification that subjects smoked > 20 cigarettes a day. According to one study, cigarette smokers can consume an estimated 13.1 mg - 22.5 mg of nicotine per day, depending on brand (Jarvis et al., 2001). The concentration of nicotine intake, therefore, varies greatly amongst subjects, as individual weights are unique. These differences in concentrations across subjects are a limitation of human studies and should be considered.

Study Limitations and Remaining Questions

The results of this study may be due to an induced upregulation and desensitization of nicotinic acetylcholine receptors (nAChR), including those on the ventrolateral preoptic nucleus (VLPO), median preoptic nucleus (MnPO), sublaterodorsal nucleus (SLD), and lateral pontine tegmentum (LPT). However, under chronic nicotine administration, not all nAChRs are desensitized (Picciotto 2007; Sparks and Pauly 2007). Future studies should specifically confirm the presence of upregulation in these brain regions, perhaps via autoradiography of α4-containing nAChRs.

Limitations of this study include a single-dose methodology and a small sample size. Future research should utilize multiple-dose methodologies and use larger sample sizes. Behavioral measures could also be studied, for behavior can indicate arousal or lethargy. These measures could provide further evidence for a dose-dependent effect of nicotine on wakefulness and sleep. Additionally, spectral analyses of EEG data should be performed. These analyses indicate the "depth" of NREM sleep that occurred. It may be that despite an increase in the percentage of time spent in NREM during nicotine administration, the NREM sleep was of poor quality. This would entail a greater portion of NREM in the light stages (alpha and theta) and a lesser portion of NREM in the deep stage (delta).

Finally, this study was researching the effects of nicotine alone. Cigarettes contain many thousands of compounds upon combustion. While not a perfect model for cigarettes, the study of nicotine is an important progression in this field.

Conclusions

This study demonstrated the effects of oral nicotine administration on female C57BL/6J mice. Nicotine affected sleep by increasing the percentage of time in NREM, decreasing the percentage of time in REM, decreasing the number of total stage shifts, and decreasing the number of sleep stage shifts. These results may have implications for nicotine addiction treatment, as treatments that exacerbate REM loss can be avoided. The improvement of sleep quality and quantity may improve abstinence success.

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References

Abd Rashid, N., Hapidin, H., Abdullah, H., Ismail, Z., & Long, I. (2017). Nicotine-prevented learning and memory impairment in REM sleep-deprived rat is modulated by DREAM protein in the hippocampus. *Brain and Behavior*, *7*(6), e00704. https://doi.org/10.1002/brb3.704

Amara, S. G., Bamberg, E., Blaustein, M. P., Grunicke, H., Jahn, R., Lederer, W. J., Miyajima, A., Murer, H., Pfanner, N., Schultz, G., & Schweiger, M. (2002). *Reviews of Physiology, Biochemistry and Pharmacology*. https://doi.org/10.1007/BFb0116584

Brown, R. E., Basheer, R., McKenna, J. T., Strecker, R. E., & McCarley, R. W. (2012). Control of sleep and wakefulness. *Physiological Reviews*, *92*(3), 1087–1187. https://doi.org/10.1152/physrev.00032.2011

Brunzell, D. H. (2008). *Neurochemistry of Nicotine Dependence. (Karch, S. B, Ed.). CRC Press.*

Centers for Disease Control and Prevention. (2019). *Current Cigarette Smoking Among Adults in the United States*. https://www.cdc.gov/tobacco/data_statistics/fact_sheets/adult_data/cig_smoking/index.htm

Dani, J. A., & Heinemann, S. (1996). Molecular and Cellular Aspects of Nicotine Abuse. *Neuron*, *16*(5), 905–908. https://doi.org/10.1016/S0896-6273(00)80112-9

Darsow, T., Booker, T. K., Piña-Crespo, J. C., & Heinemann, S. F. (2005). Exocytic Trafficking Is Required for Nicotine-induced Up-regulation of α4β2 Nicotinic Acetylcholine Receptors. *Journal of Biological Chemistry*, *280*(18), 18311–18320. https://doi.org/10.1074/jbc.M501157200

Fuller, P. M., Saper, C. B., & Lu, J. (2007). The pontine REM switch: Past and present: The pontine REM switch. *The Journal of Physiology*, *584*(3), 735–741. https://doi.org/10.1113/jphysiol.2007.140160

Gott, J. A., Liley, D. T. J., & Hobson, J. A. (2017). Towards a Functional Understanding of PGO Waves. *Frontiers in Human Neuroscience*, *11*. https://www.frontiersin.org/article/10.3389/fnhum.2017.00089

Grabus, S. D., Martin, B. R., Batman, A. M., Tyndale, R. F., Sellers, E., & Damaj, M. I. (2005). Nicotine physical dependence and tolerance in the mouse following chronic oral administration. *Psychopharmacology*, *178*(2–3), 183–192. https://doi.org/10.1007/s00213-004-2007-3

Jaehne, A., Loessl, B., Bárkai, Z., Riemann, D., & Hornyak, M. (2009). Effects of nicotine on sleep during consumption, withdrawal and replacement therapy. *Sleep Medicine Reviews*, *13*(5), 363–377. https://doi.org/10.1016/j.smrv.2008.12.003

Jarvis, M. J., Boreham, R., Primatesta, P., Feyerabend, C., & Bryant, A. (2001). Nicotine Yield From Machine-Smoked Cigarettes and Nicotine Intakes in Smokers: Evidence From a Representative Population Survey. *JNCI: Journal of the National Cancer Institute*, *93*(2), 134–138. https://doi.org/10.1093/jnci/93.2.134

Jewett, R. E., & Norton, S. (1966). Effects of some stimulant and depressant drugs on sleep cycles of cats. *Experimental Neurology*, *15*(4), 463–474. https://doi.org/10.1016/0014-4886(66)90143-9

Koehl, M., Battle, S. E., & Turek, F. W. (2003). Sleep in Female Mice: A Strain Comparison Across the Estrous Cycle. *Sleep*, *26*(3), 267–272. https://doi.org/10.1093/sleep/26.3.267

Lena, C., Popa, D., Grailhe, R., Escourrou, P., Changeux, J.-P., & Adrien, J. (n.d.). *Organization of Sleep and Regulate Putative Micro-Arousals in Mice*. 8.

Mathews, H. L., & Stitzel, J. A. (2019). The effects of oral nicotine administration and abstinence on sleep in male C57BL/6J mice. *Psychopharmacology*, *236*(4), 1335–1347. https://doi.org/10.1007/s00213-018-5139-6

Matta, S. G., Balfour, D. J., Benowitz, N. L., Boyd, R. T., Buccafusco, J. J., Caggiula, A. R., Craig, C. R., Collins, A. C., Damaj, M. I., Donny, E. C., Gardiner, P. S., Grady, S. R., Heberlein, U., Leonard, S. S., Levin, E. D., Lukas, R. J., Markou, A., Marks, M. J., McCallum, S. E., … Zirger, J. M. (2007). Guidelines on nicotine dose selection for in vivo research. *Psychopharmacology*, *190*(3), 269–319. https://doi.org/10.1007/s00213-006-0441-0

McNamara, J. P. H., Wang, J., Holiday, D. B., Warren, J. Y., Paradoa, M., Balkhi, A. M., Fernandez-Baca, J., & McCrae, C. S. (2014). Sleep disturbances associated with cigarette smoking. *Psychology, Health & Medicine*, *19*(4), 410–419. https://doi.org/10.1080/13548506.2013.832782

Mishra, A., Chaturvedi, P., Datta, S., Sinukumar, S., Joshi, P., & Garg, A. (2015). Harmful effects of nicotine. *Indian Journal of Medical and Paediatric Oncology : Official Journal of Indian Society of Medical & Paediatric Oncology*, *36*(1), 24–31. https://doi.org/10.4103/0971-5851.151771

National Institutes of Health. *Including Women and Minorities in Clinical Research Background | Office of Research on Women's Health*. (n.d.). Retrieved December 7, 2020, from https://orwh.od.nih.gov/womens-health/clinical-research-trials/nih-inclusion-policies/including-women-and-minorities

Ochoa, E. L. M., Chattopadhyay, A., & McNamee, M. G. (1989). Desensitization of the nicotinic acetylcholine receptor: Molecular mechanisms and effect of modulators. *Cellular and Molecular Neurobiology*, *9*(2), 141–178. https://doi.org/10.1007/BF00713026

Paradiso, K. G., & Steinbach, J. H. (2003). Nicotine is Highly Effective at Producing Desensitization of Rat α4β2 Neuronal Nicotinic Receptors. *The Journal of Physiology*, *553*(3), 857–871. https://doi.org/10.1113/jphysiol.2003.053447

Paterson, L. M., Nutt, D. J., & Wilson, S. J. (2011). Sleep and its disorders in translational medicine. *Journal of Psychopharmacology*, *25*(9), 1226–1234. https://doi.org/10.1177/0269881111400643

Picciotto, M., Addy, N., Mineur, Y., & Brunzell, D. (2008). It is not "either/or": Activation and desensitization of nicotinic acetylcholine receptors both contribute to behaviors related to nicotine addiction and mood. *Progress in Neurobiology*, *84*(4), 329–342. https://doi.org/10.1016/j.pneurobio.2007.12.005

Quick, M. W., & Lester, R. A. J. (2002). Desensitization of neuronal nicotinic receptors. *Journal of Neurobiology*, *53*(4), 457–478. https://doi.org/10.1002/neu.10109

Salin-Pascual, R. J., Moro-Lopez, M. L., Gonzalez-Sanchez, H., & Blanco-Centurion, C. (n.d.). *Changes in sleep after acute and repeated administration of nicotine in the rat*. 6.

Saper, C. B., Fuller, P. M., Pedersen, N. P., Lu, J., & Scammell, T. E. (2010). Sleep State Switching. *Neuron*, *68*(6), 1023–1042. https://doi.org/10.1016/j.neuron.2010.11.032

Sparks, J. A., & Pauly, J. R. (1999). Effects of continuous oral nicotine administration on brain nicotinic receptors and responsiveness to nicotine in C57Bl/6 mice. *Psychopharmacology*, *141*(2), 145–153. https://doi.org/10.1007/s002130050818

Van Dort, C. J., Zachs, D. P., Kenny, J. D., Zheng, S., Goldblum, R. R., Gelwan, N. A., Ramos, D. M., Nolan, M. A., Wang, K., Weng, F.-J., Lin, Y., Wilson, M. A., & Brown, E. N. (2015). Optogenetic activation of cholinergic neurons in the PPT or LDT induces REM sleep. *Proceedings of the National Academy of Sciences*, *112*(2), 584–589. https://doi.org/10.1073/pnas.1423136112

Vazquez, J., Guzmán-Marín, R., Salín-Pascual, R. J., & Drucker-Colín, R. (1996). Transdermal nicotine on sleep and PGO spikes. *Brain Research*, *737*(1–2), 317–320. https://doi.org/10.1016/0006-8993(96)00921-3

Watson, C. J., Baghdoyan, H. A., & Lydic, R. (2010). Neuropharmacology of Sleep and Wakefulness. *Sleep Medicine Clinics*, *5*(4), 513–528. https://doi.org/10.1016/j.jsmc.2010.08.003

World Health Organization. *Prevalence of tobacco smoking*. (n.d.). Retrieved September 27, 2020, from http://www.who.int/gho/tobacco/use/en/

Zhang, L., Samet, J., Caffo, B., & Punjabi, N. M. (2006). Cigarette Smoking and Nocturnal Sleep Architecture. *American Journal of Epidemiology*, *164*(6), 529–537. https://doi.org/10.1093/aje/kwj231

Zhang, L., Samet, J., Caffo, B., Bankman, I., & Punjabi, N. M. (2008). Power Spectral Analysis of EEG Activity During Sleep in Cigarette Smokers. *Chest*, *133*(2), 427–432. https://doi.org/10.1378/chest.07-1190