A BEHAVIORAL PARADIGM TO MEASURE NICOTINE ADDICTION IN LONG-EVANS RATS

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A Behavioral Paradigm to Measure Nicotine Addiction

In Long-Evans Rats

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To the College of Graduate Studies:

We are submitting a thesis written by Jennifer Lynn Dressler entitled "A Behavioral Paradigm to Measure Nicotine Addiction in Long-Evans Rats." We have examined the final copy of this thesis for form and content. We recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science in Community Counseling.

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Dedication

This thesis is dedicated to my children Mary (Kate), Geoff, Emily, and Adrienne. You have unwaveringly given me your love, support, and encouragement. My success is yours as much as it is mine and I thank you for joining me on this journey. I begin this new stage of life finally knowing what I want to be when I grow up. I want to be like you.

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Abstract

JENNIFER LYNN DRESSLER. A Behavioral Paradigm to Measure Nicotine Addiction in Long-Evans Rats (under the direction of Dr. Brian Hock).

Purpose: The present study investigated the use of conditioned taste aversion (CTA) to measure nicotine addiction in Long-Evans rats according to the seventh criteria set forth by the American Psychiatric Association (2000) which involves continued use of a substance despite consequences that are attributable to that substance. Once nicotine dependence has developed, cessation can be difficult and effective long-term treatments are elusive. **Method:** Thirty (30) Long-Evans rats were randomly assigned to either a control group or a treatment group with 15 in each group. For 14 days the control group received tap water while the treatment group received the 5 ug/ml nicotine solution. On the 15th day all subjects received 30 minute access to 10% blueberry juice immediately followed by a 15% lithium chloride intraperitoneal injection to invoke the CTA. Day 16 the rats were then given the choice of blueberry juice mixed with the nicotine solution or tap water. The amount of blueberry juice consumed Pre/Post CTA was measured and the discrepancy logged. The post CTA blueberry juice amounts consumed less the water consumed were also measured. The t-test was used to measure the differences between the two groups consumption for both of the two dependent variables. **Results:** The t-test for both dependent variables failed to reach significance although the treatment group did have a smaller discrepancy between the Pre/Post CTA blueberry juice consumed. **Discussion:** Schmidt et al. developed the unique approach used in this study to measure addiction. Not reaching significance may

be indicative that the low dose of nicotine or the 14 day treatment schedule was not enough to initiate nicotine addiction in the animals. **Conclusion:** A substantial body of research has investigated various aspects of nicotine dependence in people yet cessation success rates are abysmal. New motivational and behavioral models may aid in further understanding of this expensive and life threatening addiction.

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CHAPTER I

Tobacco Use and Dependence

The Department of Justice reports that approximately 25 million Americans use illicit drugs with a cost to the United States economy of a staggering $215 billion per year in "an overburdened justice system, a strained healthcare system, lost productivity, and environmental destruction" (2010, p. 3). Health care costs, lost goods and services, and lost productivity due to abuse of illicit drugs accounts for about $151 billion which includes spending on treatment, prevention, and medical consequences of abuse ($44 billion), and work loss ($107 billion) from substance related illness, incarceration, and premature death (Center for Substance Abuse Prevention (CSAP), 2009). Abuse of illicit drugs is obviously a large and expensive problem but a more pervasive and expensive situation involves the abuse of legal substances.

Prevalence and Costs. According to the most recent U. S. Census approximately 280 million people live in the United States and Puerto Rico and 21% of those people use tobacco products (U.S. Census Bureau, 2000; Centers for Disease Control (CDC), 2009). Cigarette smoking is the number one cause of preventable death in the United States (CDC, 2009). For those who smoke cigarettes nearly 58% met the criteria set forth in the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) for dependence (American Psychiatric Association (APA), 2000; Office of Applied Science, 2008). The health care costs, lost goods and services, and lost productivity due to tobacco are estimated to total $168 billion (CSAP, 2009).

The amount spent on the medical consequences of tobacco use as reported by CSAP was nearly four times greater than that spent on alcohol abuse and over 13 times greater than that spent on illicit drug abuse yet less than $1 billion was spent on preventive services (2009). Smoking and use of other tobacco products is known to contribute to numerous cancers, cardiovascular diseases, respiratory diseases, reproductive problems and other health concerns yet cessation can be difficult (Koop, 2004).

Initiation

Initiation into the smoking ranks can occur for virtually any person at any time. There are not set demographics that offer immunity. Some commonalities have been observed in those that do begin to use tobacco. Psychosocial factors play prominently in the initiation of smoking. Several studies have demonstrated that having parents, siblings, or peers who smoked increased the tendency for some to try cigarettes for the first time (Leeuw, Scholte, Sargent, Vermulst, & Engles, 2010; Oh, Heck, Dresler, Allwright, Haglund, Del Mazo, et al., 2010; Lujic, Reuter, & Netter, 2005). Of course, there were other factors investigated during these studies in relation to the propensity to smoke.

Motivation. Leeuw et al. (2010) looked at personality characteristics and social environment. Using the Big Five Personality Traits Leeuw et al. found some characteristics that were more prone to transition to smoking in certain social environments. For example, those that scored low on agreeableness were more likely

to begin smoking if a sibling smoked (Leeuw et al., 2010). Adolescents high on extraversion were more influenced by smokers in their social environment, although Leeuw et al. (2010) acknowledge that high extraversion is also associated with high thrill seeking so it may be not the former that impacts their smoking behavior but the latter. A noteworthy result involved meaningful communication between parents and their children. Regardless of the personality characteristics of the child, meaningful communication by the parents (meaningful not nagging) about cigarette smoking decreased the chances that the child would begin smoking (Leeuw et al., 2010).

Oh et al. (2010), in a study of 5000 women from 5 different countries, found the most frequently given influence for smoking initiation was having friends who smoked. The second most frequently given reason was positive reinforcement; they thought smoking looked cool (Oh et al., 2010). The association between positive and negative reinforcement expectancies on the decision to begin smoking and continuation of same was also postulated by Doran, Schweizer & Myers (2010). They discovered that the higher the beliefs in positive reinforcing effects, the earlier participants began to smoke. Starting smoking to alleviate stress or depression as well as continuation of smoking to avoid withdrawal symptoms exemplified the negative reinforcement expectancies (Oh et al., 2010; Doran et al., 2010). Doran et al. (2010) also found that those with lower reinforcement expectancies, both positive and negative, tended to maintain abstinence from smoking and their expectations did not change over time.

Education. Some advertising campaigns have attempted to counteract the beliefs in the positive reinforcing effects of smoking initiation especially in adolescents but with obviously questionable results as the adolescent smoking rates have failed to decline for the last several years after dropping for approximately the previous 30 years. (National Institute of Health (NIH), 2007). The tobacco industry funded advertising campaigns directed at children to educate and prevent the initiation of smoking by youths; unfortunately some pertinent facts were conspicuously omitted (Landman, Ling, & Glantz, 2002). The campaigns appeared to have been instituted less as a way to educate children and more as an avenue to deflect further government regulation (Landman et al., 2002). Landman et al. (2000) found that the material implemented by the tobacco industry emphasized that smoking was an adult choice, it was against the law for a minor, and blamed parents and peers for children's decision to smoke. These campaigns did not mention the dangers of smoking such as disease and addiction nor the fact that the advertising was funded by cigarette companies (Landman et al, 2000).

Advertising that addressed the fact that the previously mentioned campaigns were funded by the tobacco industry did not fare any better at promoting cessation (Rhodes, Roskos-Ewoldsen, Edison & Bradford, 2008). In fact, smokers rejected the campaigns they felt were biased or attacked the tobacco industry (Rhodes et al., 2008). They also did little to deter adolescents from transitioning from non-smoker to smoker (Rhodes et al., 2008). With that said, the 2007 report by the National Institute of Health which summarized trends for the period addressed by Rhodes et al. showed that

adolescent smoking rates declined steadily and as the funding for anti-smoking advertisements subsequently decreased so did the decline in adolescent smoking.

The anti-smoking campaigns that have been more positively received by smokers have involved those that address the dangers to others from second-hand smoke (Rhodes et al., 2008; Netemeyer, Andrews, & Burton, 2005). Netemeyer et al. found that not only did these types of anti-smoking messages positively impact the smoker's beliefs about quitting smoking; the effect was enhanced as the number of children living in the household increased (2005). Even though some have embraced the health dangers attributed to tobacco use and attempted to stop, it is unfortunate that of the 84% of those 18-24 who reported a serious attempt to quit smoking less than 9% succeeded and as the age of the smoker increased the success rate decreased. (Messer, K., Trinidad, D. R., Al-Delaimy, W. K., & Pierce, J. P. (2008).

Cessation Difficulties

Any number of factors may contribute to the difficulty experienced by those dependent on nicotine in their attempts stop using this substance. Nicotine dependence is more insidious than with some other drugs. Although there is no set period of time from the onset of smoking until dependence occurs, there is a delay which could make it easier to discount the dangers inherent to tobacco use (Breslau, Johnson, Hiripi, & Kessler, 2001). One study by Johnson, Bickel, and Baker (2007) examined delay discounting in those that have never smoked, light smokers, and heavy smokers. Comparing those that have never smoked cigarettes with the two groups of

smokers, both light smokers and heavy smokers discounted money more than their never smoked counterparts (Johnson et al., 2007). Also, both groups of smokers discounted the consequences of smoking and surprisingly all three groups discounted negative health consequences as well as positive health benefits (Johnson et al., 2007). The propensity to minimize health consequences was demonstrated by Pervin and Yatko (1965) as a means to reduce dissonance in smokers. Pervin et al. (1965) found that when smokers addressed the dangers of cigarette smoking they tended to discount reported cancer risks and minimize potential health risks to self. Only about 13% of smokers believe they will die as a result of a smoking related illness (Foss, 1973). Death due to diseases attributed to smoking was ranked by smokers as the fifth out of five choices (or least likely) to be the probable cause of their deaths (Foss, 1973). Most, nearly half, believed they were most likely die in some sort of accident long before the effects of smoking could do harm (Foss, 1973).

Contributing to the ability of smokers to delay discounting and minimize consequences could potentially be that the error processing ability in smokers appeared to be impaired (Franken, Strien, & Kuijpers, 2010). Normally when a person makes a mistake, their brain will notice the mistake so that the person can correct the error and as such, avoid making that same error in the future (Franken et al., 2010). In their study of error processing in non-smokers verses smokers, not only were smokers less proficient at noticing their errors but also at correcting for their errors and as a result, there was an impact on their decision making (Franken et al., 2010).

A study by Versace, Robinson, Lam, Minnix, Brown, Cater, et al. (2010) addressed attention in smokers who are dependent and trying to abstain from smoking. Smokers can become accustomed to smoking cues that signal the brain that it is time to smoke and as such cravings begin as well as provoking an emotional response in the smoker (Versace et al., 2010). As a result of these cues and the subsequent craving induced by these cues the smokers' attention becomes so focused on calming the craving that it becomes an automatic response (Versace et al., 2010). Due to limited attention resources available during withdrawal, smokers have difficultly focusing on activities that could distract from the craving and lead to healthy alternative activities (Versace et al., 2010).

Versace et al.'s findings coincided with the argument that nicotine dependence was more the result of operant conditioning and the repetition of the physiological movement and this factor played prominently in the impaired ability to discontinue the habit (Lujic et al., 2005). Girard, Turcotte, Bouchard, & Girard (2009) studied participants in a smoking cessation program that involved participants crushing cigarettes in a virtual reality setting. They used a 12 week program with the treatment group crushing cigarettes while the control group manipulated balls in virtual reality. They found a significant difference between the treatment group and the control group in continued dependence, abstinence, and retention in the program. It was possible that the results attained were due to a new conditioned response to the smoking cues that incited cravings (Girard et al., 2009). Participants in the treatment group demonstrated higher motivation as well as enhanced self-efficacy in their ability to

abstain from smoking (Girard et al., 2009). Interestingly, a closer review of the data revealed that the treatment group consistently reported cybersickness (feeling ill due to the virtual reality simulator) yet that was not considered as a possible factor in the higher success rates.

Women and cessation. In relation to smoking cessation, there are other factors that may play into the difficulties experienced by women when trying to quit smoking. It has been shown that self identity plays a role in successful smoking cessation (van den Putte, Yzer, Willemsen, & de Bruijn, 2009). Those whose self-identity as that of 'smoker' and who identified themselves as like other smokers, found quitting smoking more difficult. For women, not only can cigarette smoking impact their self-identity, but their perception of their own femininity as well (Gilbert, 2007).

Purportedly, the number of male smokers in movies and television has decreased in the last few decades yet, according to Gilbert (2007), the portrayal of women smokers in the media has not only increased but smoking is shown in a favorable light. This coupled with tobacco companies designing cigarettes and packs focused toward women, has lead young women to believe their very gender identity was connected with smoking (Gilbert, 2007).

Weight gain was identified as another issue for women, especially when quitting smoking. Weight related issues were reported by the CDC (2009) as the number 2 preventable cause of death in the United States and young women already have a higher propensity to gain weight (Wane, van Uffelen, & Brown, 2010) so cessation for

women has been even more challenging. Pomerleau, Zucker, & Stewart (2001) found that women used smoking as a way to maintain their weight and were concerned about weight gain if they quit smoking. Fear of weight gain has been a deterrent to cessation attempts in women, including pregnant women, and was associated with relapse (Janzon, Hedblad, Berglund, & Engstrom, 2004; Berg, Park, Chang, & Rigotti, 2008). These findings were more prevalent in Caucasian women than in other races possibly due to varying ideal body image (Pomerleau et al., 2001). Interestingly, although the phenomenon of weight gain was also found in older, post-menopausal women upon quitting smoking, muscle mass also increased which may lead to improved mobility (Kleppinger, Litt, Kenny, & Oncken, 2010).

Smoking and Alcohol

Smoking maybe the number one preventable killer but alcohol is number three (CDC, 2009) and all too frequently these substances are combined. Approximately 80 to 95% of those that are alcohol dependent also smoke cigarettes and 70% are heavy smokers (National Institute on Alcohol Abuse and Alcoholism (NIAAA), 1998). A study by Le, Lo, Harding, Juzytsch, Marinelli, & Funk (2010) demonstrated that alcohol and nicotine seem to reinforce each other and after a period of abstinence from both substances, reintroducing either nicotine or alcohol causes a reinstatement of both. The potential for nicotine and alcohol to work together to keep an aversion to either substance from developing, as well as creating withdrawal symptoms that deter

discontinuation of either substance was found, although the exact mechanisms involved were not discovered (Onaivi, Todd, & Martin, 1989).

Genetics and Dependence

The mapping of the human genome was completed just after the turn of this century and was lauded as the profound way to revolutionize prevention, diagnosis, and treatment (Collins & McKusick, 2001). Unfortunately, this has yet to be realized. What was discovered, specifically in relation to substance abuse and dependence, was potentially hundreds of genes were involved with numerous external factors and no clear way to determine what factors would cause which genes to activate (Sellman, 2009).

An extraordinarily rudimentary explanation of dependence as related to the brain involves use of a substance that illicit a hedonic reaction by the brain. Repeated use of that substance causes the reward to be lessened due to those mechanisms in the brain becoming desensitized and attempting to regain homeostasis. To achieve the same pleasurable sensation or to avoid the symptoms of withdrawal more of the substance must be introduced. With that said, not all who indulge in an addictive substance become dependent. Just as it has been demonstrated that alcohol dependence can involve a genetic component, the same can be said of nicotine (Kalant, 2009). As no single genetic trait, but a combination of several genetic traits, when acted upon by other factors, create a vulnerability that can cause dependence to occur (Kalant, 2009). Kalant posited that it was environmental factors, in conjunction with the

voluntary use of the substance by those genetically predisposed, that accounted for the development of dependence (2009).

Sellman (2009) agreed that dependence is about 50% heredity along with too many environmental factors to count but moved the voluntary use in to the realm of compulsion. With nearly three-quarters of those who attained treatment for substance dependence also having another psychiatric disorder, Sellman (2009) argued that it was the compulsion that created the dependence and the inordinate relapse rates. Agrawal and Lynskey (2008) attributed the persistence of nicotine dependence back to heredity at a rate ranging from 50% to 70% with little impact from the environment. To bring this argument full circle a study by Chassin, Presson, Seo, Sherman, Macy, Wirth et al. (2008) demonstrated that currently smoking parents did in fact significantly impact smoking in adolescents compared to formerly smoking parents but the age of initiation for both groups of parents appeared to influence the results.

Transition to College

Research has suggested that as educational levels increased, smoking rates tended to decrease (Breslau et al., 2001). These results appeared to reinforce the findings by Colder, Flay, Segawa, Hedeker, & TERN (2008) members that overall the rate of smoking decreased as students move through their undergraduate degree. A study conducted by Warner, Halpern, & Giovino in 1994 showed the perceived dangers of smoking was not greater in those with the highest levels of education compared to the lowest levels of education. More current studies on this topic were not found during

the literature review. Colder et al. (2008) did find a connection between increased or continued smoking for those with smoking peer groups. When alcohol was added to the social situation rates of smoking increased even more for those that were normally considered light smokers (Jackson, Colby, & Sher, 2010).

There is little surprise that in a report issued by the American Society of Addiction Medicine, "Nicotine dependence is the most common form of chemical dependence in the United States [and] is especially prevalent among those who suffer from alcoholism" (1996, p. 1). Rates of smoking are not only higher in relation to alcoholism but also in recovering drug users, HIV patients, and the mentally ill (Dunn, Sigmon, Reimann, Badger, Heil, & Higgins, 2010; Harris, 2010; Grant, Hasin, Chou, Stinson, & Dawson, 2004).

Animal Models of Addiction

Numerous animal models have been developed to better understand the effects, causes, and continuation of addiction. The transition to smoking frequently occurs during adolescence and much earlier than one might think; the early teen years. (NIH, 2007). In a study of adolescent rat nicotine use, Shram & Le (2009) used intermittent exposure over a very short period of time. The results demonstrated that adolescent rats, compared to adult rats, were more sensitive to nicotine, received greater rewarding effects at lower doses, and developed a conditioned place preference in only four sessions. (Shram et al., 2009). After cessation of the nicotine exposure and extinction of the conditioned place preference in adolescence, re-exposure to nicotine

immediately reinstated the conditioned place preference, reinforcing the rewarding effects of the nicotine on the adolescent rats (Shram et al., 2009).

The adolescent brain is in a state of transition and development. When nicotine was introduced into those developing neuronal pathways Riley, Zalud & Diaz-Granados (2010) observed changes to the development. Alcohol was thought to work on some of the same receptors as nicotine and it was possible the changes in the neurochemical pathways due to early nicotine use, especially GABA and NMDA, caused an altered response to alcohol in adulthood (Riley et al., 2010). This altered brain function, possibly caused by the nicotine use in adolescence, was found to create more serious withdrawal from alcohol dependency in adulthood (Riley et al., 2010). Strangely Riley et al. discovered that when nicotine and alcohol were both introduced in adolescence the same withdrawal phenomenon in adulthood was not observed (2010).

Drugs, including nicotine, have been demonstrated in rats to have significantly different effects in the reward and reinforcement attained by gender (Yararbas, Keser, Kanit, & Pogun, 2010). Yararbas et al. (2010) used multiple levels of nicotine to determine if there was a difference in drug seeking at different levels depending on sex. Male rats spent significantly longer in the chamber paired with the nicotine at each level (Yararbas et al., 2010). Only at the highest dose of nicotine did female rats spend more time in the nicotine paired chamber and it was still not significant in relation to the amount of time spent in the non-treatment chamber (Yararbas et al., 2010). Yararbas et al. (2010) also tested the premise that the anxiolytic effect was the reason for nicotine

consumption as opposed to the rewarding effects. This was not the case for either the male or female subjects.

During the early stages of drug taking behavior, the substance is used more sporadically. To measure the effects of intermittent, acute use and resulting withdrawal Engelmann, Radke, & Gewirtz (2009) used a measurement of anxiety to assess severity of withdrawal symptoms. Initially the rats were injected with nicotine, the withdrawal observed, and then re-injected with nicotine (Engelmann et al., 2009). They measured the withdrawal in relation to the dose of the drug. Engelmann et al. (2009) then introduced a drug to counteract the withdrawal symptoms.

The results of the study showed that repeated exposure to nicotine increased each subsequent withdrawal until the halfway point in the experiment (Engelmann et al., 2009). During the last half of the study the withdrawal symptoms began to decrease in the treatment group and increase in the control group (Engelmann et al., 2009). These changes in the respective stress responses were attributed to the impending injections that both groups repeatedly received throughout the study (Engelmann et al., 2009). The habitual injections that created more anxiety in the control group were believed to have been counteracted in the treatment group by the nicotine (Engelmann et al., 2009).

Jackson, Kota, Martin, & Damaj (2009) also studied the withdrawal syndrome, using mice, including differences by age and gender. This research measured avoidance due to being made ill. The results substantiated a difference by age and gender in

nicotine withdrawal in relation to conditioned place aversion (Jackson, et al., 2009). The animals involved developed an aversion that was only maintained through the first two conditioning sessions (Jackson et al., 2009). By the third session adaptation had occurred and the aversion was no longer great enough to deter the conditioned place aversion to nicotine (Jackson et al., 2009). When different antagonists were used to create the aversion, evidence suggested that the nicotine more strongly influenced the central nicotinic receptors but not the peripheral ones (Jackson et al., 2009). These results added further validation that the central nicotinic receptors are responsible for affective withdrawal signs (Jackson et al., 2009). Male subjects were impacted by the conditioned place aversion whereas the female subjects showed much less aversion possibly demonstrating that females are less sensitive to the symptoms of withdrawal (Jackson et al., 2009). Adolescent animals demonstrated results similar to those of the female animals although the subjects were not separated by gender (Jackson et al., 2009).

As a caveat to the central nicotinic receptor theory expressed by the previously cited study, Grabus, Martin, Batman, Tyndale, Sellers, & Damaj (2005) had previously studied dependence and tolerance with nicotine. The Jackson et al. (2009) study used mecamylamine with dihydro-B-erythroidine as the antagonist and stated the response witnessed showed the central nicotinic receptors were involved in nicotine addiction. Grabus et al. (2005) also used mecamylamine and noted only this substance but not the other antagonists initiated the withdrawal demonstrating that nicotine influences the

central and the peripheral nicotinic receptors. For virtually each discovery about nicotine addiction another study was available to offer in rebuttal.

The American Psychiatric Association (APA, 2000) uses seven criteria to address the issue of substance dependence: tolerance; withdrawal; extended or increased use; failed attempts to quit; excessive time spent to attain, use, or recover from the substance; social impairment; and continued use despite adverse consequences. Dependence has most frequently been measured by the first two criteria set forth by the APA (2000), tolerance and withdrawal. The present study used the model developed by Schmidt, Schmidt, & Hock (2008) as an avenue to measure nicotine addiction in Long-Evans rats from the seventh criteria, continued use despite adverse consequences (APA, 2000). The difficulties associated with tolerance and withdrawal from higher doses of nicotine have been previously investigated, as cited within the present study. This study investigated the hypothesis that rats will become addicted to nicotine, even at a low dose, and because of that addiction, when compared to the control group, will continue to consume more of the substance that previously made them ill.

CHAPTER II

Method

Subjects

Thirty (30) Long-Evans (Harlan) rats 120 days old were used for this study. The animals were housed individually in Plexiglas cages in the animal vivarium on a 15:9 light/dark schedule. They were given 7 days to acclimate before initiation of the study. The animals were randomly assigned into one of two groups; the control group or the experimental group, with 15 rats in each group. The animals had access to food *ad libitum* but were water restricted 23 hours per day with the exception noted below. Their daily treatments were administered through test tubes with drinking spouts. Full IACUC approval was received before the outset of this study.

Design and Procedure

Twenty-four hours before the start of the experiment all rats were water deprived. Beginning on day one, the experimental group of 15 rats was given 20 mls of 5 ug/ml nicotine (Sigma) mixed with water while the 15 rats in the control group were given 20 mls of tap water. Both groups were allowed access to the fluid for 30 minutes which was delivered via graduated test tubes with drinking spouts. The amount of fluid consumed by each rat was subsequently measured and recorded. This process continued for a total of 14 days. On the 15th day both groups of rats were given a 30 minute exposure to 20 mls of 10% blueberry juice (Wal-Mart) immediately followed by

an intraperitoneal (IP) injection of 0.15 M lithium chloride (Carolina Biological Supply) at 2.0% of their free feeding body weight to induce a conditioned taste aversion. On the 16th day both groups were given 30 minute access to a choice of 20 mls of both the 10% blueberry juice mixed with the 5 ug/ml oral nicotine solution or a second test tube containing plain tap water. To measure the conditioned taste aversion and the addiction, two dependent variables were used. The first dependent variable measured if there was a reduction of 10% blueberry juice drinking from before verses after the injection. The second dependent variable measured the animal's preference for the blueberry juice with nicotine minus the water.

CHAPTER III

Results

The one independent variable used for this study, prior to the CTA, was whether the animals received plain tap water (control group) or the nicotine solution (treatment group). Pre-CTA/Post-CTA difference score in blueberry juice consumption and the preference for blueberry juice mixed with the nicotine solution minus the water consumed were the two dependent measures for this study. One subject from the treatment group was excluded from analysis due to failure to receive the full measured dose of lithium chloride at test (n=14).

The mean consumption of fluids, measured in milliliters, during days 1 – 14 for the control group (M=13.12, SD=8.47) and the treatment group (M=13.97, SD=1.67) showed no significant difference ($t(26)=0.37$, $p> .10$). Furthermore, the amount of blueberry juice consumed Pre CTA by the control group (M=15.58, SD=2.26) and treatment group (M=15.50, SD=1.19) also showed no significant difference ($t(27)=-0.05$, $p> .10$). To ensure that the rats were not being impacted by a place preference of the bottles, the water bottles and blueberry/nicotine solution bottles were alternated in position in every other placement. There was no significant difference in the amounts of either the water or the blueberry/nicotine solution by placement.

Although the treatment group did consume more blueberry juice solution after the CTA, the independent-measures t-test performed to measure the Pre/Post CTA difference for the blueberry juice consumed, comparing the amounts consumed by the

control group (M= -9.107, SD= 8.815) and the treatment group (M= -10.80, SD= 13.394) failed to reach significance ($t(27)=1.36$, $p>.05$). The preference for the blueberry juice mixed with the nicotine solution less the water consumed also failed to reach significance ($t(27)=-0.049$, $p>.05$) between the control group (M= -6.179, SD= 37.446) and the treatment group (M= -6.05, SD= 59.538).

Nicotine has previously demonstrated to be addictive, the present study failed to initiate an addiction in the rats. Although the research on nicotine addiction in animals that was previously cited in this study used higher levels of nicotine, they also used a longer duration The dose of nicotine was low (5 ug/ml) and as a result a longer duration than the 14 days used for this study may be necessary to achieve nicotine addiction.

It is incontrovertible that nicotine is addictive yet the present study failed to initiate an addiction in the rats. Previous research on nicotine addiction in animals used higher levels of nicotine and exposures that were longer than 14 days, the duration of this study (Andreasen, Nielsen, & Redrobe, 2009; Gaddnas, Pietila, & Ahtee, 2000). The present study used a low dose of nicotine (5 ug/ml) and although 14 days has been shown to be an adequate time for addiction to a substance to commence (Schmidt et al., 2008), it is possible that a longer exposure was necessary to induce addiction at such a low dose of nicotine.

CHAPTER IV

Discussion

Schmidt et al. (2008) explored addiction using the American Psychiatric Association's (2000) seventh criteria which states that dependent users will continue to use the substance with knowledge that the adverse consequences were likely a result of the substance. The Schmidt et al. (2008) model provided a cost effective and efficient method to examine addiction in animals. The use of the Schmidt et al. (2008) model allowed the present study an avenue to measure nicotine addiction in rats.

The results of the this study failed to reach significance which could indicate that either lower levels of nicotine or relatively short exposures or a combination of the two could delay the onset of nicotine addiction. The previous statement notwithstanding, there was indication that the discrepancy between the amounts of blueberry juice consumed before verses after the CTA was smaller in the treatment group. This may be denotative that an addiction in the rats had begun to develop.

The present study did not involve a progressive ration of nicotine nor a constant exposure. The rats received a 30 minute oral exposure once daily to a very weak dose 5 ug/ml of nicotine. Other studies that have used oral exposure to nicotine used 10 to 100 times stronger solutions (Andreasen et al., 2009; Gaddnas et al., 2000). In these studies the nicotine was paired with a sweetener due to the purportedly offensive taste of the nicotine. It has been postulated that rats will not consume nicotine at a dose of 5ug/ml or higher because of aversive nature of the taste and subsequent digestive upset

(Flynn, Webster, & Ksir, 1989). The present study did not pair the nicotine with a sweetener and no significant differences were found in daily intake of fluids between the water only group and nicotine group.

Limitation of this Study

The most obvious limitation to this study involved the use of oral nicotine verses inhaled nicotine. A single daily exposure to oral nicotine by the animals is a different dynamic to the sporadic and repeated inhaled nicotine of a cigarette smoker. It has been postulated that nicotine inhalation is essential to addiction (Schneider, Olmstead, Franzon, & Lunell, 2001), the studies previously cited are offered in rebuttal. Although inhalation delivers nicotine to the blood stream very quickly, oral and nasal ingestions also raise the levels in the body and nicotine is consumed by humans in a variety of methods and not limited to inhalation. Nicotine levels in the animals were not measured and as such plasma levels may not have reached a significant level to induce addiction.

Other possible limitations lay in the nicotine solution and duration of exposure. The solution administered to the experimental group was comprised of 5 ug/ml of nicotine/water and constituted a weak dose of nicotine. The potency of the nicotine for this study was a discretionary amount used as a starting point to measure addiction at low levels of exposure. Previous studies involving mice have used nicotine solutions that were 10 to 100 times stronger than those used for this study (Andreasen et al., 2009; Gaddnas et al., 2000). Both Andreasen et al. (2009) and Gaddnas et al. (2000)

used increasing amounts of nicotine solution over a longer period of time to ensure that blood nicotine levels reached that of a daily cigarette smoker. Although the time frame of 14 days has been shown to initiate addiction (Schmidt et al., 1977), the delay in addiction that was associated with nicotine (Breslau et al., 2001) could be greater than that used in this study. It is possible that the duration of the present study was not long enough to initiate addiction.

Implications for Future Research

Additional information that adds to the behavioral models of addiction in animal and subsequently dependence in humans is essential for creation of cessation plans that are more effective. More importantly, development of educational programs that deter smoking initiation would save millions in medical and societal expenses. Replication of this study with slight adjustments to the potency of the nicotine solution or constant exposure as opposed to limited exposure may demonstrate that the rats in this study were in fact at the cusp of addiction. Adjustments to the duration of the experiment might also be a valid approach to gleaning more information. If in fact the reason the treatment group consumed more blueberry juice after the CTA than the control group was that they were in the initial stages of addiction this may demonstrate that even at very low doses addiction can develop in a relatively short period of time and add to the body of information on nicotine addiction.

CHAPTER V

Conclusion

The years of research on smoking and tobacco use has continually proven that nicotine use is expensive, dangerous, and addictive yet finding effective long-term interventions seems to remain elusive. The act of smoking and potential dependence in humans is an amalgamation of not only ingestion of nicotine but also the psychological habit and the physical act of using the tobacco; handling the cigarette or chewing tobacco, the hand to mouth movement, crushing the cigarette, and so forth. While the present study cannot address every aspect of addiction it may provide information toward the motivational factors involved in nicotine addiction as well as other addictions.

The investigation of addiction has involved genetic components, motivations, behavior changes, withdraw difficulties, tolerance, personality types, and a plethora of other concerns yet, as evidenced by the numerous studies previously addresses here, little progress has occurred toward successful cessation. Throughout this literature review only two propositions have been even somewhat definitive: Dependence cannot occur unless the substance is first used and cessation, once dependence has occurred, is extremely difficult. Better methods to keep adolescents and adults from initiating nicotine use are needed. For those that are already dependent, potential treatments need to be improved because currently successful long term abstinence, at a rate of over 60%, has only been achieved by intensive medical counseling with one group;

those that have had a myocardial infarction, better known as a heart attack (U. S. Public Health Service, 1979).

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