THE EFFECT OF NICOTINE WITHDRAWAL-INDUCED STRESS ON CHRONIC ANXIETY IN ADOLESCENCE AND ADULTHOOD

by

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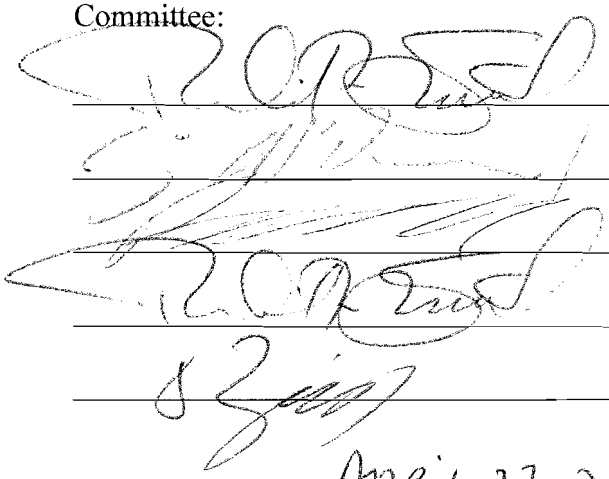

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The Effect of Nicotine Withdrawal-induced Stress on Chronic Anxiety in Adolescence and Adulthood

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Arts at George Mason University

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DEDICATION

This is dedicated to my mother and father, who love and support me no matter what the cause.

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I would like to thank Dr. Robert Smith, Dr. Craig McDonald, and Dr. Patrick McKnight for their dedication and guidance throughout the thesis process. I would also like to thank my lab mates: Kelsey Brown, Erin Beech, Erica Holliday and Gina Fernandez for their invaluable help in the preparation of this project.

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ABSTRACT

THE EFFECT OF NICOTINE WITHDRAWAL-INDUCED STRESS ON CHRONIC ANXIETY IN ADOLESCENCE AND ADULTHOOD

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Tobacco users generally start their habit during adolescence; 80-90% of tobacco-using adults report that they had their first tobacco experience before they turned 18 (Manhaes, Guthierrez, Filgueiras, & Abreu-Villaca, 2008). According to Manhaes et al. (2008), evidence found early in adolescence indicates that the brain of an adolescent is easily more susceptible to gaining and maintaining a strong dependence on tobacco. Smoking during adolescence is associated with future use that is typically chronic and this usage lessens the likelihood of decreased nicotine consumption over time. There are many factors that play a role in adolescent nicotine use. Anxiety is known to be a crucial factor for initiating nicotine use because of the motivating factor to continue consumption due to elevated anxiety. Although nicotine temporarily alleviates anxiety for tobacco users, increased anxiety is also a symptom of tobacco withdrawal (Manhaes et al., 2008). The differences between adolescent and adult nicotine consumption differ greatly. This study sought to understand these anxiety differences through withdrawal symptoms associated

with cessation of chronic nicotine use. This study was essential in order to better understand how nicotine consumption illustrates and modifies behavior after withdrawal symptoms occur. Adults and adolescents were divided into eight groups: adolescent/adult saline familiar, adolescent/adult saline unfamiliar, adolescent/adult nicotine familiar, adolescent/adult nicotine unfamiliar.. The nicotine was administered to nicotine withdrawal groups at 4.7 mg/kg for adolescents and 3.2 mg/kg for adults for 7 days via osmotic minipumps to promote chronic nicotine addiction. A dose of 1.5 mg/kg for adolescents and 3.0 mg/kg for adults of mecamylamine was given to all groups during EPM testing and on OF trial day 3 to test the effects of withdrawal-induced stress that promotes anxiety-like behavior. Once tested, adolescents showed no significant differences when environment type was manipulated. Adolescents exhibited less anxiety-like behavior during withdrawal on the OF task. Even when exposed to a novel environment, adolescents still maintained a lower amount of anxiety-like behavior than adults.This further supported the notion that adolescents differ in their reactions to withdrawal when compared to adults.

1. INTRODUCTION

Tobacco users generally start their habit during adolescence; 80-90% of tobacco-using adults report that they had their first tobacco experience before they turned 18 (Manhaes, Guthierrez, Filgueiras, & Abreu-Villaca, 2008). According to Manhaes et al. (2008), evidence found early in adolescence indicates that the brain of an adolescent is easily more susceptible to gaining and maintaining a strong dependence on tobacco. Smoking during adolescence is associated with future use that is typically chronic and this usage lessens the likelihood of decreased nicotine consumption over time. There are many factors that play a role in adolescent nicotine use. Anxiety is known to be a crucial factor for initiating nicotine use because of the motivating factor to continue consumption due to elevated anxiety. Although nicotine temporarily alleviates anxiety for tobacco users, increased anxiety is also a symptom of tobacco withdrawal (Manhaes et al., 2008).

Slawecki, Thorsell, El Khoury, Mathe, & Ehlers (2005) reported that adolescent smokers were found to have a higher anxiety level than adolescents who do not smoke. It was also reported that adolescents that have higher anxiety were more likely to rely on tobacco to cope with their constant stress. Some studies have shown that adolescent rats that have been exposed to nicotine are not as likely to approach food placed in the middle of an open field test while a conflict test is being administered (Slawecki et al., 2005).

These findings indicate not only anxiety-related behaviors but also depressive symptoms as well in adolescent rats. This supports the hypothesis that nicotine exposure during adolescence could induce anxiety or depressive behaviors which continue into adulthood. The elevated plus maze (EPM) is a model used to measure anxiety in the rodent population (Slawecki et al., 2005). Decreased time in the open arms of the apparatus and decreased open arm entries observed in rats that have been exposed to nicotine show an increase in anxiety-like behaviors. Slawecki et al. (2005) found that the total number of arm entries in the EPM was gradually reduced in rats that had been exposed to nicotine. It was also found that decreased activity levels (decrease open arm entries) may confound the measures used to report anxiety-like behavior in the elevated plus maze.

Many recent animal studies report and confirm that adolescent nicotine exposure alters neurobehavioral actions such as anxiety-like behaviors as well as brain system development (Slawecki, Gilder, Roth, & Ehlers 2003). Slawecki et al. (2003) states that adolescent nicotine exposure has been shown to produce hippocampal and cortical cell loss and increase nicotinic receptor binding in the hippocampus and cortex; This shows that adolescent nicotine exposure has neurobehavioral effects that may last into adulthood. A study conducted by Slawecki et al. (2003) to assess increased anxiety-like behavior in adolescent rats showed that in the standard open field test adolescent rats that were administered nicotine had a significantly lesser amount of perimeter square entries as well as total square entries.

According to Elliot, Faraday, Phillips, & Grunberg (2004), in studies using a variety of adult male mammals, nicotine has been shown to have no effect on behaviors

related to anxiety. This finding predicts that there may be a metabolic difference in the way that adolescents and adults are affected by nicotine. A study done by Elliot et al. (2004) using the EPM showed an increase of time spent in the open arms and this was seen as proof of anxiolysis, and it was found that adolescents spent more time in the open arms than adult rats. It was also found that nicotine treated animals spent less time in the open arms than saline treated animals. Among adolescent groups, nicotine exposure increased the amount of time spent in the open arms for males but decreased for females (Elliot et al., 2004). When all of the animals were analyzed together, adolescent rats had a higher percentage of open arms entries than adult rats did. With adult rats, nicotine exposure reduced the percentage of open arm entries with all of the groups differing significantly from one another. This study showed that adolescent males are more sensitive to nicotine exposure's anxiety-relieving effects than adolescent females and adult males and females. These effects, while shown, may depend a great deal on the context in which the anxiety occurred (Elliot et al., 2004).

While there is emphasis placed on determining the affects of nicotine at a neurochemical level, it is also important to consider the environment and its potential to influence the withdrawal effects of animals. A study was conducted by Hamilton, Berger, Perry, & Grunberg (2009) that tested withdrawal effects in rats during young adulthood (P70) by observing their withdrawal symptoms in different environment types. The different environments included a dimly-lit room with bedding in their cages and a brightly lit room with no bedding in their cages. The observed behaviors during withdrawal are based on a model conducted by the Malin group, this model has been used

in many studies and has had positive results. The model consists of the following behavioral symptoms of withdrawal: whole body shakes, abnormal grooming, abnormal posture or movement, ptosis, empty-mouth chewing/teeth chattering, eye blinks, and diarrhea (Hamilton et. al., 2009). The male and female rats were observed by two different trained researchers four different times overnight to assess the behavioral patterns of the rats before, during and after the nicotine was administered. After seven days, nicotine was no longer given via osmotic pump and the rats were assessed for their withdrawal symptoms 20 hours after the nicotine was removed and then 24 hours after the nicotine had been removed from their systems. Their locomotor activity was measured for one hour using the Open Field Test to measure for differing activity. This was done for experiment one: dimly lit, with bedding and for experiment two: brightly lit, no bedding, for both the males and the females (Hamilton et. al., 2009).

There were three major findings in this experiment: nicotine withdrawal does exist and can be modeled in rodents, nicotine withdrawal exists in both sexes, and the environment has an effect how nicotine withdrawal symptoms are expressed, especially in males (Hamilton et. al., 2009). Males that had received nicotine showed greater withdrawal behaviors in a brightly-lit environment than in a dimly lit environment. Females that had received nicotine showed similar amounts of withdrawal symptoms in both the environments. It was found that differences between saline and nicotine groups were not the result of differences in locomotor activity. It was recently reported that nicotine withdrawal is a result of the corticotropin-releasing factor (CRF) system activating the CRF1 receptors that facilitate anxiety-like behavior. Additive stressors

during withdrawal may promote the system and eliminate withdrawal. In the study done by Hamilton et. al. (2009), nicotine withdrawal could have been effected by environmental stress, especially in male rats.

Many debates have been conducted regarding the use of different anxiety tests using animal models. It is often found that multiple tests should be used to account for environmental differences and to see if effects can be found using the different environments. The EPM, OF, and Light-Dark box or conditioned-place preference tests are all used to measure anxiety and addiction in animal models. Ramos (2008) asks: Do we need to use more than one test when screening for drugs and other effects? Animal tests are based on behaviors that depend on activity of the body and locomotion. The EPM is the first choice when testing anxiolytic drugs and is based on the conflict between exploring a new environment and avoiding a possibly dangerous environment (Ramos, 2008). Acute stressors can be influential on the behavior seen during the EPM such as anxiety from electric shock, surgical stress, saline injection, forced swim, social defeat, cat odor, and other anxiety related symptoms which can reduce the amount of exploration in the EPM (Hogg, 1996). Hogg (1996) found that in some cases even increasing light levels has led to avoidance of the open arms on the EPM. Walsh and Cummins (1976) report that high levels of illumination are connected to diminished locomotor behavior in the OF. Some researchers believe that all the measures of these paradigms are interrelated. Other researchers have proof that this is not the case: chlordiazepoxide produced anxiolytic effects in the EPM but not in the OF (Ramos, 2008). In addition to

the tachykinin NK1 receptor antagonist in hypersensitive rats with chlordiazepoxide, anxiolysis was present in the OF but not the EPM (Ramos, 2008).

. While the measure of the apparatuses ability to measure differences in anxiety has been questioned by some researchers, furthermore is the question of what types of results are found when comparing adolescent and adult anxiety-like behaviors in different apparatuses. There are findings that conclude that adolescents are more prone to nicotine dependence and find even low doses rewarding (Smith, McDonald, Bergstrom, Brielmaier, Eppolito, Wheeler, Falco, & Smith, 2006). Adults, on the other hand, are not as susceptible to nicotine and do not perceive low doses as rewarding as adolescent rats do. There are many reports that indicate lasting behavioral and biological effects of nicotine exposure that are specific to the adolescent time frame (Smith et.al., 2006). Upregulation of the nicotinic cholinergic receptors in the brain occurs as well as an increased desire for nicotine into adulthood and decreased reward involving cocaine in adulthood. Many of these studies suggest that there are certain features that are unique to the adolescent brain and that these differences are related to plasticity. Open field test studies have shown that anxiety-like behavior is present in adult male Sprague-Dawley rats given nicotine during adolescence as shown by lessened locomotor activity (Smith et. al., 2006). On the other hand, hyperactivity has been reported in the open field test regarding adult males exposed to nicotine during adolescence. Lessened food-oriented behavior after adolescent exposure to nicotine has also been documented and is an indicator of lasting anxiety-like behaviors (Smith et. al., 2006).

In the study conducted by Smith et. al. (2006) male and female rats were broken down into three groups: a control group, a low nicotine group and a high nicotine group. All rats received the doses during adolescence or adulthood via osmotic pump for 14 consecutive days. The rats were then tested for differences in the locomotor behavior in the open field test and were tested for extinction/fear conditioning one month after the end of their nicotine dosing period. This study showed that the adult exposure groups with the same amount of abstinence from nicotine, showed prominent and lasting effects in open field behavior that are specific to nicotine exposure during adolescence (Smith et. al., 2006). This suggests lasting anxiogenic effects after adolescent nicotine exposure. Smith et. al.'s (2006) study provides evidence and promotes the notion of the following: adolescents who use nicotine chronically during adolescence are anxious as adults when nicotine is not presented. The lower doses of regular nicotine during adolescence enhance fear learning and complicate fear extinction during adulthood which further promotes the evidence of an anxious emotional state of being. This study furthers my and many other researchers belief that adolescence is a critical time involving addiction and the recurrent behavioral changes that occur because of it during adulthood. It seems that if these behavioral changes continue into adulthood that the likelihood of the behavioral symptoms being alleviated without the drug or a safer replacement is slim to none at this current point in time.

It has been hypothesized based on animal studies that when compared to adults, adolescents have short-term positive effects and reduced aversive effects during withdrawal when using nicotine (O'Dell, 2009). During adolescence, these strong

positive effects are balanced by negative effects that lead to the increased dependence into adulthood. Dopamine in increased in the nucleus accumbens when nicotine is ingested and is lessened during the withdrawal period. During adolescence excitatory glutaminergic systems are overdeveloped and these help to increase dopamine when nicotine is ingested. The inhibitory GABAergic systems are underdeveloped during adolescence.

According to O'Dell (2009):

> Adolescents display enhanced nicotine reward and reduced withdrawal via enhanced excitation and reduced inhibition of the ventral tegmental area cell bodies that release dopamine in the nucleus accumbens. This review suggests that clinical diagnostic criteria developed for nicotine dependence in adults, based primarily on withdrawal, may be inappropriate during adolescence when nicotine withdrawal does not appear to play a major role in nicotine use. (p. 263)

The understanding of the progression of nicotine dependence from adolescence to adulthood is the key. According to O'Dell (2009), certain treatment strategies and replacements for nicotine may be detrimental for adolescents and heighten the vulnerability to nicotine dependence in adulthood.

The aim of this study was to determine the effects of nicotine withdrawal on chronic anxiety-like behvavior between adolescence and adulthood. This study sought to determine if there were differences and similarities related to a novel environment versus a familiar environment when testing chronic anxiety-like behavior across both age groups. This has given insight into how anxiousness works in both age groups as opposed

to just comparing the effects of nicotine on anxiousness. It produced a framework for possible environments in which anxiety-like behavior related to nicotine withdrawal may be more prevalent. This design has shown how much these effects are heightened or shadowed depending on the environment and the age group. This has shed light on the amount of difference between both age groups and how much anxiety-like behavior related to withdrawal effects the group.

I hypothesized that since the EPM was novel to all groups that the adult groups would exhibit more anxiety-like behavior overall than the adult nicotine groups. I also hypothesized that the adult groups exposed to nicotine and exposed to the familiar environment upon withdrawal would be the most anxious. It seems that adolescents would also exhibit more anxiety-like behavior once withdrawal occurred and especially if they were exposed to the novel environment while going through withdrawal. However, due to the nature of the adolescent brain, I hypothesized that this behavioral effect would not be great compared to the behavioral effect it would have on adults. Furthermore, it was important to know whether or not the environment made a difference when going through withdrawal as it has been shown to in a human model.

2. METHODS

2.1 Subjects

Male Sprague- Dawley rats weighing 35-49 grams at postnatal day 21 and 250-300 grams at postnatal day 52 were obtained from Harlan Laboratories (Indianapolis, IN). The animals arrived one week before testing began and were subject to individual handling by the experimenter for a one minute period two times a week to reduce handling stress during experimentation. Animals were housed in groups of five on a 12 hour light/ dark schedule. The animals had ad libitum access to food and water throughout the experiement.

2.2 Experimental Group Assignments

There was a total of eight experimental groups, each with 10-12 animals per group. An a priori power analysis concluded that a total of 96 animals were needed to find a moderate effect size. Animals were housed according to their group identfication which were as follows:Adolescent-Saline-Familiar; Adolescent-Saline-Unfamiliar; Adolescent-Nicotine-Familiar; Adolescent-Nicotine-Unfamiliar; Adult-Saline-Familiar; Adult-Saline-Unfamiliar; Adult-Nicotine-Familiar; Adult-Nicotine-Unfamiliar. The groups assignments were based on age, drug treatment, and environment type. The familiar environment was considered exposure to the OF on Trial Days 1, 2, and 3 while the unfamiliar environment was considered exposure to the OF only on Trial Day 3. The

Elevated Plus maze (Kinder Scientific, Poway, CA) was arranged in a plus configuration with two arms (50.2×10.8 cm) enclosed by 40-cm high black Plexiglas walls and two opposed open arms (50.2×10.8 cm). The entire maze was elevated 86 cm above the floor. To avoid influencing behavior, extra-maze cues in the room were minimized and light levels in the room were ~25 lx. At the start of the trial, the rat was placed in the center of the apparatus facing a closed arm and allowed to explore the maze for 5 min. Activity in the maze was recorded by overhead camera. Videos were hand scored by the experimenter. Total time in the open arms versus the closed arms was measured in seconds. Animals received 10-minute trial(s) during testing in the open field apparatus (42 x 42 x 30 cm3). Center time (17.8 x 17.8 cm2) was measured (Viewpoint, Montreal, QC, Canada). A solution of 70% EtOH was used between animals on the EPM and OF to clean the apparatus. Behavioral scores were averaged in two 5-minute intervals.

2.3 Materials

Nicotine hydrogen tartrate was purchased from Sigma Chemical Company (St. Louis, MO). Saline and nicotine were administered via Alzet osmotic mini pumps (Durect Corp., Cupertino, CA, USA). Following anesthesia with ketamine xylazine (anesthetic dose based on individual weight), the lower back was shaved and a small incision made to permit implantation. A single 14-day pump (Model 2002) was implanted in the adolescent animals (p28) and in the adult animals (p59). Nicotine dose (free base) was 4.7 mg/kg over a period of two weeks for adolescents and 3.2 mg/kg over a period of two weeks for adults. The nicotine was distributed to the assigned groups. The saline groups received 0.9% saline solution in the pumps. Despite being marked as a 14-

day pump, infusion was continuous since this model does not become exhausted until at least Day 18 (calculations based on manufacturer information).

2.4 Procedure

Animals began testing on the EPM prior to the removal of mini pumps All groups were tested on the EPM on Trial Day 1 for initial reaction to a novel surrounding to assess how this effected withdrawal-induced anxiety. EPM testing was used to determine what type of an effect mecamylamine had behaviorally when a sensitive one-trial test was administered with no environmental manipulations. After mecamylamine (1.5 mg/kg, intraperitoneal injection) was induced each animal (sham and withdrawal and nicotine adolescent and adult groups) was allotted 10 minutes time before being placed on the EPM for 5 minutes. The open arm time in seconds and the closed arm time in seconds was recorded for each animal. After EPM testing, animals were be given a washout period of 48 hours before being tested on the OF. The 48 hour washout period allowed time for the mecamylamine to leave the body (Sietse, Jonkman, Risbrough, Victoria B., Geyer, Mark A., & Markou, Athina 2008). The serum elimination half-life of mecamylamine is approximately 1 hour (Debruyne, D., Sobrio, F., Hinschberger, A., Camsonne, R., Coquerel A., & Barre, L. 2003).

Following EPM testing and after the washout period, OF was conducted. The OF test was used in order to test the anxiety-like withdrawal effects via environmental manipulation. The familiar groups were tested on the OF during trial days 1, 2 and 3. The animals were allotted 20 minutes to become habituated to the testing room containing the OF apparatus. The animals were then placed in the OF for 10 minutes at a

time and center time was recorded as well as the pathway during time spent in the OF. On the 3rd trial day, the familiar and unfamiliar groups were tested to assess the difference that the environment type can make with regard to anxiety-like withdrawal effects.

Design Outline

Week Three

-EPM testing was conducted on all animals. Mecamylamine was administered to determine withdrawal effects on all animals.

-Animals were given a 48 hour wash out period.

-Open Field testing was administered on trial days 1, 2, and 3. Familiar groups were tested on all three trial days. Unfamiliar groups were tested only on trial day 3. All animals were exposed to mecamylamine only on trial day 3.

3. HYPOTHESIS

Previous work has demonstrated that the adolescent brain is susceptible to continued drug consumption due to the developing brains reward circuit. This study sought to determine how adolescents and adult react behaviorally in different environment types when exposed to a chronic amount of nicotine and placed in a familiar or novel environment. The Elevated Plus maze and the Open Field apparatus were used in order to determine how the animals would be effected behaviorally. I hypothesized that since the EPM would be novel to all groups that the adult nicotine group would be more anxious overall than the adolescent saline groups. This hypothesis was determined due the finding that adults are biologically designed to react greater to the absence of nicotine in regard to anxiety-like behavior. I also hypothesized that the adult group exposed to the familiar environment upon withdrawal would exhibit the most anxiety-like behavior.

It seemed that adolescents would be more anxious once withdrawal occurred and especially if they were exposed to the novel environment while going through withdrawal. However, due to the nature of the adolescent brain, I hypothesized that this behavioral effect would not be great compared to the behavioral effect it would have on adults. Furthermore, was important to know whether or not the environment made a difference when going through withdrawal as it has been shown to in a human model.

4. RESULTS

4.1 Statistical Analysis

To determine the effect of the chronic nicotine dosage in all animal groups a 2 x 2 ANOVA (age: adolescent, adult; drug: saline, nicotine) was used to determine if there was a significant interaction on the third day of exposure for the familiar group and the first day of exposure to the unfamiliar group when both were given mecamylamine.

4.2 Open Field Withdrawal

There was a significant main effect of withdrawal between the groups, F (51, 49) = 63.3, p = .01. Mean comparisons illustrate that adult animals conditioned with nicotine had significantly lower difference scores which means that less time was spent in the center, (M= 39.5) than adult animals conditioned with saline (M= 87.1). These results signify that the adult animals treated with nicotine that received mecamylamine on trial Day 3 showed more anxiety-like behavior by spending less time in the center than the adult saline animals treated with mecamylamine. The adolescent animals treated with saline were found to show significantly less anxiety-like behavior than those treated with nicotine. There was an interaction between the age and drug variables when inducing withdrawal-induced anxiety. Adults experienced withdrawal-induced anxiety-like

behavior across drug while adolescents experienced the opposite effect. Main effects and the interaction are illustrated in figure 1.

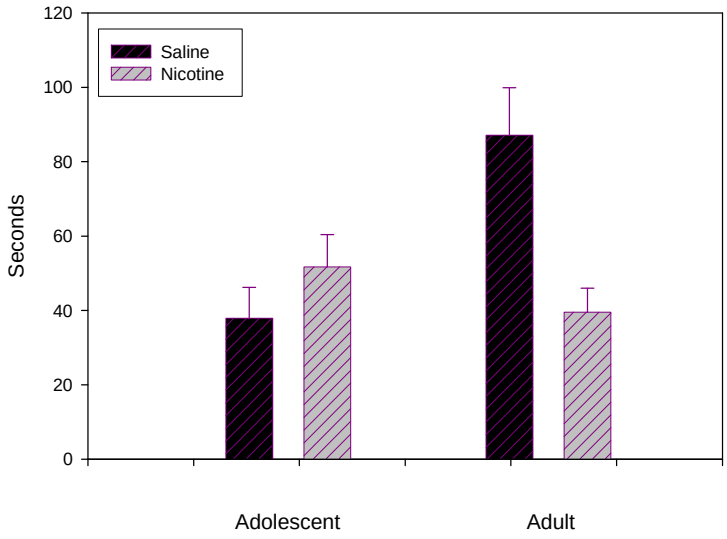

Figure 1 Center time scores by age and drug. Adolescents were less anxious during mecamylamine-induced withdrawal than adults. Saline-treated adolescent animals were increasingly more anxious than saline-treated adult animals. $p < 0.05$, on OF trial day 3

There was no significant interaction effect between familiar and unfamiliar groups between age, F (51, 49), p = .523. The graph below illustrates that there were no differences between environment type. Adolescents were found to show significantly more anxiety-like behavior than adults when exposed to the familiar environment on trial day 3 with F (51, 49) = 27.2, p = .026. Adults showed no significant effects. Effects are illustrated in figure 2.

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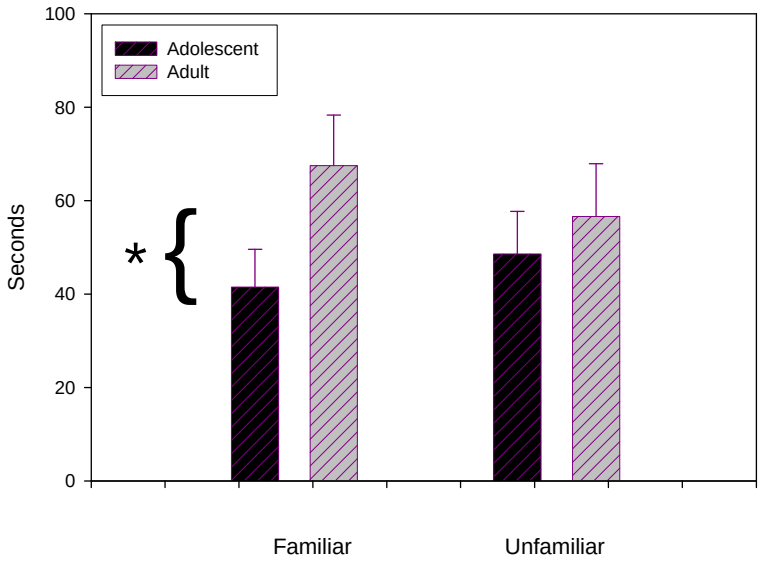

Figure 2. Difference scores on OF trial day 3. Time spent in the center between age and environment type, measured as decreases in difference scores, varied across age groups in the familiar environment (adolescent or adult) but not for environment type (familiar or unfamiliar) on OF trial day 3, $p < 0.05$.

4.3 Elevated Plus Maze

T-tests were run to compare the differences between adolescents and adults exposed to saline and nicotine. These tests also sought to determine if there was a significant difference between age and drug. A proportion of OAT/CAT was calculated. The greater the proportion calculation, the more time that was spent in the open arm compared to the closed arm. It was determined that adolescents exhibited slightly more anxiety-like behavior during withdrawal while adults showed less anxiety-like behavior. The similarities in behavior between ages could be due to behavioral manipulation by

mecamylamine. Comparisons determined that all the EPM data were insignificant p = .939 as illustrated in figure 3

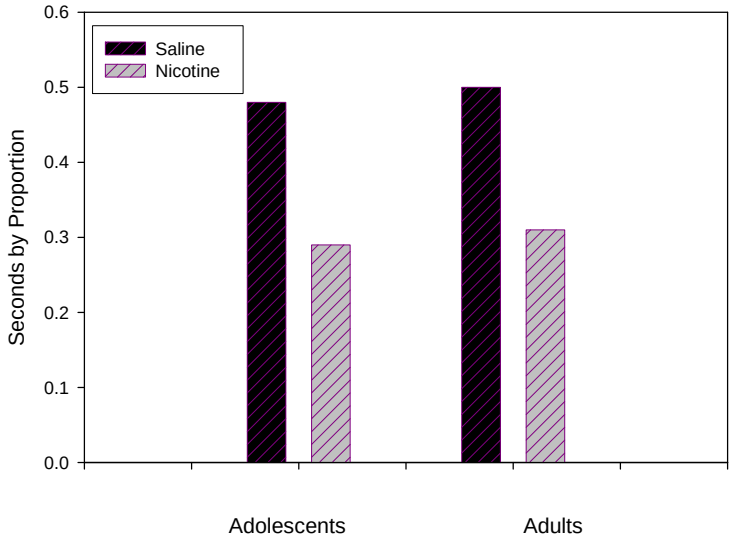

Figure 3. Time spent in the OAT/CAT by drug, measured in seconds, barely varies across age (adolescent or adult) during mecamylamine-induced withdrawal, *p = .939.*

5. CONCLUSIONS

The results of this study found that adolescents showed less anxiety-like behavior during withdrawal when tested in the OF. Adolescent saline-treated animals exhibited more anxiety-like behavior than adult saline-treated animals. Adolescents showed no significant differences when environment type was manipulated (familiar or unfamiliar). During EPM testing, adolescents had slightly less anxiety-like behavior during withdrawal but it was not significant. O'Dell (2009) stated that adolescents display enhanced reward and reduced withdrawal due to enhanced excitation and reduced inhibition in the brain. This statement holds true. Clear differences have been found that determine that nicotine and withdrawal effect adolescents and adults differently. This difference is due to biological mechanisms but is also tied to the motivational aspect of behavior toward a novel environment between the age groups. This paper sought to test anxiety-like behavior due to meca-induced withdrawal in manipulated environment types in order to assess the effect(s) that it had across groups. Since withdrawal does not play a major role in nicotine use during adolescence, it was only fitting that adolescents would be less anxious overall (O'Dell 2009). This study gave insight into how withdrawal affects anxiety-like behavior and how environment did not seem to play a role.

Changes in the animals behavior could be due to the suggestion that mecamylamine causes anxiolytic properties under stressful conditions (Newman, Nazian,

Sanberg, Diamond, & Shytle 2001). This could have countered the anxiousness associated with withdrawal related behavior. A study conducted by Trauth, Seidler, & Slotkin (2000) looked into behavior in a novel open field paradigm that was assessed during post natal days 30-47 and for two weeks later post treatment. Two weeks after cessation of nicotine administration males were unaffected when it came to locomotor deficits and were unaffected when it came to grooming behavior compared to females. In adolescent males, nicotinic receptor upregulation lasts longer. Male adolescent rats also show a suppression of hippocampal cholinergic activity that continues even after nicotine cessation. It seems that males may be less susceptible to nicotine-induced effects. While males do not show differences under basal conditions, there are differences seen when exposing males to behavioral learning tasks. It has been suggested that in order to determine the effects of adolescent nicotine exposure on the brain, a learning or integrated response task may be needed.

Many factors can contribute to the accidental manipulation of certain environmental and biological aspects when testing. Adolescence is an especially crucial time when any form of manipulation, intentional or not, can greatly affect the outcome of any measure taken. In a study conducted by Abreu-Villaca, Queiroz-Gomes, Dal Monte, Filgueiras, and Manhaes (2006), adolescent rats (PND 30) were exposed to a hole board activity box. Their novelty-seeking behavior and anxiety levels were assessed. The number of head dips was used to divide the groups into high novelty seeking or low novelty seeking groups. It seems that the HN animals consumed more nicotine than the LN animals. This suggests that the animals that wanted to explore the new environment

more had high curiosity about doing so and were more enticed to consume greater amounts of nicotine. When anxiety was measured depending on the percentage of center squares crossed, it was found that anxiety levels had no effect on nicotine consumption. Since adolescence is associated with a greater motivation to seek out new stimuli when exploring novel environments, this may provide some insight into why the environment types had no effect in the OF and why the mecamylamine seems to have lessened the amount of anxiety seen in the nicotine groups behaviors. The higher the motivation to seek out a new environment in adolescence, the more likely it is that drug use will occur and anxiety is not a major contributor that decides differential nicotine consumption in adolescence.

Nicotine has been shown to have anxiolytic effects when studies have been done between sexes. This information is pertinent because males have shown a greater sensitivity to the anxiolytic effects of nicotine than females. In a study conducted by Cheeta, Irvine, Tucci, Sandhu & File (2001) male and female adolescent rats were divided into groups labeled isolation or socially housed. It seems that the anxiolytic effect was seen only in socially isolated animals. Locomotor effects were also lessened in males which could cause males to be less likely to explore a novel environment. While nicotine increased social interaction in males and took a less amount of nicotine than in females, this just adds yet another factor into the list of effects that can count toward trying to elicit differences between adolescents and adults with regard to withdrawal effects and anxiety.

Nicotine enhances attention while nicotine withdrawal can lead to attentional deficits. One debate that has occurred many times is whether nicotine actually improves performance or reduces attentional deficits. Semenova, Stolerman, & Markou (2007) tested acute and chronic nicotine administration as well as withdrawal on rats using a 5-choice serial reaction time task. Acute nicotine administration induced small increases in speed of responding and impulsivity. Chronic nicotine administration increased accuracy. Nicotine withdrawal showed more decreases in correct responses and increases in latency to respond. This study showed that chronic nicotine induced improvement with regard to accuracy and that nicotine withdrawal led to limited performance deficits. This study further supports the notion that withdrawal has only detrimental effects on animals. While chronic nicotine can support learning to a degree, the cost of withdrawal carries a great weight with regard to consuming a large amount of nicotine to maintain reinforcement of these additional properties.

A study done by Kwilasz, Harris, & Vann (2009) compared the somatic and behavioral effects in regard to withdrawal. Mice were trained to press a lever for food during daily operant sessions. Mice were then given different doses of nicotine via osmotic mini pumps. Somatic signs of withdrawal were assessed after the administration of mecamylamine and also after spontaneous removal of nicotine. Somatic signs increased in mice while withdrawal did not decrease operant responding. Mecamylamine did not produce signs of withdrawal in either procedure. Nicotine dependence in mice during spontaneous removal of nicotine was shown. Since no signs of behavioral withdrawal were observed, this study provides insight into how important it is to consider

differences in withdrawal differentiation when trying to evaluate nicotine dependence. This study ties together the many variables that have to be considered when deciding to readily manipulate and elicit withdrawal effects to determine what type of effect it has across age, environment type or even sex.

In conclusion, these findings suggested that while differences were seen between adolescents and adults with regard to drug exposure: saline or nicotine, differences were found with regard to withdrawal. All the different factors discussed above could impact the effect that nicotine and mecamylamine had and what effects were manipulated by the environment. While the EPM data suggested that mecamylamine played a role in lessening the rate of anxiety-like behavior once withdrawal occurred, the OF data further reiterated the conclusion that there were significant differences in withdrawal-induced anxiety-like behavior between adolescents and adults. How adolescents and adults reacted to withdrawal was so strong that they can be elicited when an anxiolytic drug is administered. While the environment did not have an effect in this design, this study helped to give insight into the strength and differences between how adolescents and adults reacted to mecamylamine-induced withdrawal.

APPENDIX

A study conducted by Manhaes et al. (2008) showed that after a three day withdrawal period, anxiogenic effects in both sexes of adolescent rats were observed. After another two days, females were still influenced by the anxiogenic effects of withdrawal from nicotine. These different behavioral responses show that males and females may react differently regarding the time course of their responses to nicotine. This study suggests that anxiety level changes during withdrawal periods may be the behavioral basis of differences in functioning in the activity of the cholinergic systems due to nicotine exposure during adolescence.

Manhaes et al. (2008) reported the following:

> The nicotinic cholinergic receptors were shown to modulate anxiety which indicates that nicotine effects anxiety through the cholinergic system. The activation of pre-synaptic nicotinic receptors plays a neuromodulatory role in the central nervous system promoting neurotransmitter release and affecting other neurotransmitter systems. Adolescent nicotine exposure produces increased nicotinic receptor binding, impaired cholinergic activity and serotonergic synaptic function as well as cell loss in the hippocampus. (p. 222)

It has been shown that exposure to minimal amounts of stress which activates anxiety also activates the brains corticotrophin-releasing factor and neuropeptide Y systems which are important factors and mediators of anxiety and depression (Slawecki et al., 2005). Acute foot shock stress increases cortical CRF release in the brain. Slawecki et al. (2005) found that in the forced swim test, there was a longer latency pattern when becoming immobile and a decreased amount of immobility was seen in adolescent rats exposed to nicotine. Escape attempts were higher in adolescent rats exposed to nicotine during the FST which contradicts previous studies reporting decreased activity in adolescent rats exposed to nicotine. In this study it was found that the behavior elicited by the FST is related to already existing anxiety-like behavior in rats that have been exposed to nicotine during adolescence (Slawecki et al., 2005).

Smoking during adolescence is correlated with an increase in a greater amount of tobacco dependence, a higher daily consumption rate and a lowered probability of ever quitting the habit (Wimouth & Spear, 2006). Many neurochemical changes are found when chronic nicotine usage begins during adolescence such as: death of cells in the cerebreal cortex, midbrain and hippocampus. There are also alterations in the synaptic functions of serotonin, the cholinergic and the catecholaminergic pathways in the brain. The upregulation is more persistent and occurs in a different regional pattern for nicotinic acetycholine receptors in adolescent rats (Wilmouth & Spear, 2006). Withdrawal-induced anxiety has been considered an important factor when contributing to continued smoking in humans and this has been modeled successfully using rats on the EPM test.

According to Wilmouth & Spear (2006), when rats were assessed 18 to 24 hours after nicotine withdrawal, the animals that had been chronically treated with nicotine demonstrated an increase in anxiety-like behavior. This is measured by a decrease in the time spent in the open arms of the EPM when compared with saline-treated control rats. The EPM is highly sensitive to retest effects but given adolescent rats' sensitivity to the stimulating and reinforcing effects of nicotine, it was anticipated that adolescents would be likely to show a higher sensitivity to nicotine withdrawal symptoms (Wilmouth & Spear, 2006).

In the study discussed above by Wilmouth & Spear (2006), withdrawal-related anxiogenesis was not found in adolescents but was found in adults when tested on the EPM. Dopamine levels that are reduced within the nucleus accumbens are believed to play a major role in mediating nicotine withdrawal (Wilmouth & Spear, 2006). This could reflect ontogenetic immaturity in systems that cause an inhibition of irrelevant sensory and cognitive information processing and this could cause a reduction in stimulus filtering during withdrawal periods for nicotine-dependent adolescents. In the study conducted by Wilmouth & Spear (2006), adolescents were found to be unaffected by withdrawal effects of nicotine while adolescent smokers report anxiety as the most prevalent withdrawal symptom that is experienced by humans next to cravings. This data suggests that adolescents should be less likely to become dependent on nicotine. Adolescent animals were found to be more sensitive to nicotine when assessed for withdrawal effects using the prepulse inhibition of the acoustic startle response (PPI). This suggests that cognitive interference could contribute to rapid dependence during adolescence despite low daily exposure to nicotine during adolescence (Wilmouth & Spear, 2006).

Few studies have compared the behavior of adolescent and adult rats on tests used to observe and collect anxiety-like responses. There are three kinds of traditional anxiety-measuring animal tests which are the Elevated Test, the Open Field test, and the Elevated Plus Maze. All of these apparatuses measure anxiety through the use of a novel environment for the animal which elicits a certain amount of stress in itself. According to Lynn & Brown (2010), adult female rodents have been reported to show a higher locomotor rate in the OF, to spend more time in the open arms of the EPM, and to emerge sooner in the ET than adult male rodents. Also, in the EPM, adolescent female rats have been shown to exhibit more open arm activity than male rats of the same age. The measure of the study done by Lynn & Brown (2010) focused on the difference between male and female rats in four stages of maturity: adolescence (pnd 24-39), late adolescence (pnd 51-55), young adulthood (pnd 65-69) and older adulthood (pnd 104-109). The OF test showed that there was a significant effect of sex with females having more locomotor activity than males. In the EPM, there was a significant effect of age on the amount of time spent in the open arms. The trend revealed that the percentage of time in the open arms increased linearly with age. The percentage of time spent in the open arms of the EPM positively correlated with all of the locomotor measures of the OF (Lynn & Brown, 2010).

The results of this study indicate that the amount of anxiety-like behavior and exploratory behavior lessen across all age groups of the study on all three behavioral tests

(Lynn & Brown, 2010). It was in this study conducted by Lynn & Brown (2010) that showed that the time spent on the open arms and the number of open arm entries on the EPM increased from adolescence to adulthood. The results do not support the theory that adolescent rodents are more exploratory in nature and less anxious than adults. This study showed that adolescent rats do spend less time on the open arms than adults when using the apparatus that was scaled to their size. It seems like anxiety-like responses are similar for all three types of apparatuses though females did show higher motivation than males for investigating/escaping novel environments (Lynn & Brown, 2010).

A growing number of studies have shown that stress exposure has a longer lasting effect on adolescent rodents rather than adult rodents. Previous research has also shown that EPM performance is sensitive to prior testing in another apparatus and that pretest manipulation can reduce the differences of sex differences in EPM results (Lynn & Brown, 2010). The main conclusion is that anxiety-like behavior is shown to decrease from adolescence into adulthood in rats. It seems that while novel environment induced anxiety lessens with age, it is extremely and increasingly more important for behavioral tests involving administered drugs like nicotine to be administered and tested. This way the different behavioral effects of the drug can be analyzed over time and be compared to the results of novel environment effects of anxiety. Studies involving drug effects and anxiety will continue to be done due to the importance of assessing the drug efficacy and behavioral effects of that efficacy.

The potential to abuse nicotine is suggestively linked to its reinforcing and rewarding components that also involve the activation of the mesocorticolimbic dopamine system of the brain which is suggested to be the neural substrate that underlies drug self-administration in animals used for experiments (Molander & Soderpalm, 2003). Regular administration of nicotine has been shown to increase burst firing and regular firing activity of dopamine neurons. This results in increased dopamine output in the nucleus accumbens. According to Molander & Soderpalm (2003), evidence shows that behavioral and reinforcing stimulant effects of nicotine are greatly influenced by environmental factors but mostly by stress and stress hormones. In this study, it was found that acute amounts of nicotine administered to rats showed that the animals habituated to the testing apparatus (activity boxes) and increased their locomotor activity. This effect was sensitized after a daily pretreatment of 15 days in which nicotine was administered. Locomotor stimulatory effects after nicotine consumption are due to dopamine receptors activating in the nucleus accumbens (Molander & Soderpalm, 2003).

Sensitization of this effect has been shown to involve an enhancement of dopamine-releasing effects of nicotine and also a sensitivity involving postsynaptic dopamine receptors (Molander & Soderpalm, 2003). After repeated nicotine administration, the D1 and D2 dopamine receptors were increased and there was a sensitization seen involving locomotor stimulatory effects involving dopamine release in the brain. These results show that locomotor sensitization may come from post synaptic rather than presynaptic hyperactivity of the mesocorticolimbic dopamine system. In the study by Molander & Soderpalm (2003), an adrenalectomy was conducted on the animals to measure the effects of ablating this gland. The study showed that dopamine levels after

nicotine administration were not altered by the adrenalectomy therefore concluding that endogenous corticosterone is not involved in the dopamine liberating effect.

The adrenalectomy did prevent the behavioral sensitization to nicotine and is correlated with the postsynaptic side of the dopamine system. This study indicates that behavioral sensitization to nicotine is associated with stimulatory locomotor effects of the D1/D2 receptor agonist apomorphine and is likely to involve dopamine receptors in the nucleus accumbens. Molander & Soderpalm (2003) concluded that the adrenal glands appear to promote postsynaptic sensitization of the mesolimbic dopamine system in response to nicotine exposure that is repeated. This study indicates the importance of understanding the sensitization of behavioral effects at a neurochemical level to better understand the pathways and neurotransmitters involved in the manipulation and administration of nicotine's behavioral effects.

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