DEVELOPMENT OF ANXIETY LIKE BEHAVIOR IN THE RAT

by

Shealyn G. Holt

A Thesis
Submitted to the
Graduate Faculty
of
George Mason University
in Partial Fulfillment of
The Requirements for the Degree
of
Master of Arts
Psychology

Committee:

_____ Director

_____

_____

_____ Department Chairperson

_____ Dean, College of Humanities and Social Sciences

Date: December 7, 2011

Fall Semester 2011
George Mason University
Fairfax, VA

Development of Anxiety-Like Behavior in the Rat

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

By

Shealyn G. Holt
Bachelor of Science
Villanova University, 2005

Director: Bob Smith, Professor
Department of Psychology

Fall Semester 2011
George Mason University
Fairfax, VA

DEDICATION

This is dedicated to my parents, Debra Coleman, Lue Gene T. Holt, and John Winston Holt, whose unwavering love and support made this thesis possible.

ACKNOWLEDGEMENTS

This dissertation would not have been possible without the guidance and the help of several individuals who in one way or another contributed their valuable assistance in the preparation and completion of this study.

First and foremost, my sincere gratitude to my advisor Dr. Robert F. Smith, whose steadfast encouragement and guidance from my initial acceptance into the program to the final level of my education, enabled me to develop my understanding of biopsychology and the nature of animal research.

Dr. Craig McDonald, for his tireless hours spent reviewing the validity of the study and fostering the development of my analytical skills.

Dr. Adam Winsler, for graciously serving as a member of my thesis committee offering his patience and keen attention to detail.

I have considered it an honor to work with my classmates in the Biopsychology department, including Dan Ehlinger, Jennifer Brielmaier Sontag, and Adriana Falco and graciously thank them for their help and assistance in conducting the study.

Last but not least, my family and friends, without whose unwavering love and support, this thesis would not be possible. I am indebted to my mother who always sacrificed so that I may succeed, my grandmother, who has always been a constant source of support and strength, my grandfather for instilling within me drive and ambition to achieve, you are loved beyond imagination and missed beyond belief, and to my nieces who directed me in my life's passion and taught me that no matter how difficult things may seem, giving up is never an option. Finally, I am ever thankful for my partner in crime for his constant support of achieving my goals.

TABLE OF CONTENTS

Page

List of Figures……..vi
Abstract……viii
Introduction……1
Review of the Literature ……3
Methods……10
Participants ……10
Apparatus ……10
Open Field Apparatus ……11
Elevated Plus Maze……12
Viewpoint Tracking ……13
Statistical Analyses ……13
Results……14
Open Field Apparatus ……14
Total distance traveled ……14
Elevated Plus Maze……15
Percentage of time on open arms ……15
Percentage of open arm entries ……15
Latency to enter the open arms ……16
Discussion……18
Conclusion ……21
References……23

LIST OF FIGURES

Figure Page

1. Elevated Plus Maze 12
2. Total distance traveled in the open field apparatus 13
3. Percentage time spent in the open arms of the elevated plus maze 14
4. Percentage entry into open arms of the elevated plus maze 15
5. Latency to enter the open arms of the elevated plus maze 16

ABSTRACT

DEVELOPMENT OF ANXIETY-LIKE BEHAVIOR IN THE RAT

Shealyn G. Holt, M.A.

George Mason University, 2011

Thesis Director: Dr. Robert F. Smith

Age has been shown as one variable that influences anxiety-like behavior in both human and animal models. Adolescence is a time of major brain growth and development, which underscores the importance of investigating emotional development during this time period. While little research has examined behavioral development during adolescence in animal models, there are data suggesting that anxiety and susceptibility to drug effects are related. The present study investigates the effect of age on anxiety-like behavior in rats, specifically examining seven time periods throughout the course of adolescence into adulthood, with analysis on four separate age groups in early adolescence, mid-adolescence, late adolescence, and adulthood. The results indicated that anxiety-like behavior is a function of age, with early adolescent and adult animals exhibiting higher levels of anxiety than that of their mid-adolescent and late-adolescent counterparts. More specifically, animals in the p28-p32 age cohort, when compared with the p36-p40 and p44-p48 age cohort, exhibited increased anxiety levels in total distance traveled in the

open-field apparatus, percentage of time spent on the open arms of the elevated plus maze, as well as the percentage of entries into the open arms of the elevated plus maze. The results of this study may have important implications for future studies due to the fact that the development of neurobiological systems and emotions continue throughout adolescence and into adulthood, and the development of these systems may help understand future drug use and addiction models.

INTRODUCTION

Age has been shown as one variable that influences anxiety, depression, and addiction in both human and animal models. The fact that adolescence, in particular, is a time of major brain growth and development makes it important to investigate emotional development and its implications for drug use and addiction during this life stage. Late childhood and early adolescence has been marked as a time period in which experimentation with drugs begin (Brown & Tapert 2004; Johnston, O'Malley, Bachman, & Schulenberg, 2005; Smith & Morrell, 2007; Spear 2000; Turner, Mermelstein, & Flay 2004), and mental health disorders, such as anxiety, begin to surface (Smith & Morrell 2007). Spear has documented that the neurobiological systems are still undergoing major changes during the period of adolescence and may be affected by the use of pharmacological drugs (Dahl & Spear, 2004). Further literature has documented that adolescent intake of drugs may alter the development of the central nervous system to the point where adult functioning may be modified due to adolescent drug exposure (Smith, 2003).

The time period of adolescence is marked by a series of transitions into adulthood, with multiple physiological changes taking place. Therefore, it is difficult to characterize a single event that marks the beginning and end of adolescence, particularly with the limited amount of animal adolescent research (Spear, 2000). Due to the inability to

absolutely define the constraints of adolescence, animal research has loosely defined the time period into a conservative age range of postnatal days p28-p42 (Spear, 2000). During this time frame there is a developmental peak in the N-methyl-D-asparate (NMDA) receptor binding, rats emerge from their protected nests in the wild, and the vaginal opening in females and maturing spermatids in males are seen (Spear, 2000). Further animal research indentifies adolescence as a series of time periods falling into the following categories: early adolescence, p27-p30; late adolescence, p38-p41, and adult p90-p99 (Belluzzi, Lee, Oliff, & Leslie, 2004). During this period, the hypothalamic pituitary adrenal axis (HPA) is also undergoing maturational changes (Anderson, 2003; Lynn & Brown, 2010; Romeo, 2005). These developmental changes alter the hormone levels in both the gonadal and adrenal glands, potentially increasing the likelihood that adolescents may develop an anxiety disorder (Anderson, 2003; Lynn & Brown, 2010; Romeo, 2005).

REVIEW OF THE LITERATURE

Recent studies have begun looking at the important correlation between anxiety and depressive disorders and their links with addiction, withdrawal, and relapse (Weinberger, Desai, & McKee, 2010). It has been well documented that individuals with a current mood disorder or anxiety disorder are more likely to report withdrawal symptoms than those without current mood disorders (Weinberger et al., 2010). Anxiety and depressive disorders have also been associated with an increased likelihood of withdrawal-related discomfort and relapse (Weinberger et al., 2010). Depression and anxiety symptoms have significant effects on addiction behavior, as seen in alcohol and nicotine studies. Heavy drinkers with co-occurring depressive and anxiety symptoms exhibit increased alcohol use in greater quantities and demonstrate a greater vulnerability to relapse than those individuals without co-occurring depressive and anxiety symptoms (Buckner, Timpano, Zvolensky, Sachs-Ericsson, & Schmidt, 2008; Feldstein Ewisng, Filbey, Chandler, & Hutchinson, 2010; Grotheus, Bischof, Reinhardt, Meyer, John, & Rumpf, 2008; Kushner,Abrams, Thuras, Hanson, Brekke, & Sletten, 2005).

In response to clinical research conducted on human participants, depressive and anxiety symptoms have also been investigated in laboratory animal studies of alcohol cue-elicited craving (Breese et al., 2005; Chiang, Schuetz, & Soyka, 2005; Cooney, Litt, Morse, Bauer, & Gaupp, 1997; Feldstein Ewing et al., 2010; Fox, Berquist, Hong, &

Sinha, 2007; Litt, Cooney, & Morse, 2000; Sinha, 2007; Sinha, Fox, Hong, Berquist, Bhagwagar, & Siedlarz, 2008). In laboratory studies of cue reactivity, a participant is exposed to drug-related paraphernalia, such as cigarettes or alcohol, as well as neutral cues, such as rulers or notebooks. Following the presentation of the stimulus, physiological reactions, drug-use behaviors, and self-reports are analyzed. While there is some debate about the strength of the relationship between cue reactivity associated with relapse, the majority of studies have exhibited a compelling relationship between craving, depressive symptoms, and anxiety symptoms among heavy drinkers (Breese et al., 2005; Chaing et al., 2005; Cooney et al., 1997; Feldstein Ewing et al., 2010; Fox et al., 2007; Litt et al., 2000; Sinha, 2007; Sinha et al., 2008).

While there is a clear relationship between anxiety and addiction, developmental age as a variable has been well-studied only for reactions to addictive drugs. Conditioned place preference studies are commonly used to evaluate the addiction potential of certain drugs. Within a laboratory setting, animals are presented with a positive stimulus, such as nicotine, along with distinct environments with different visual cues, such as light versus dark. When the animal is tested again in a normal state, the amount of time spent in the light or dark environment, paired with the positive stimulus is evaluated. Increased amounts of time in environments associated with the positive stimulus are associated with the drug reward. Conditioned place preference studies conducted with nicotine as the positive stimulus have shown that a single nicotine injection induced a significant place preference in adolescent rats when compared with late adolescent and adult rodent counterparts (Belluzzi et al., 2004). Further research conducted in conditioned place

preference apparatus models showed that adolescent animals (p28) who were pretreated with a single injection of .5 mg/kg of nicotine, showed lasting tolerance (one month later) to the effects the injection at .5mg/kg while the adults showed tolerance to the .1mg/kg dose ((Brielmaier, McDonald, & Smith, 2007), suggesting that age and drug susceptibility are related.

Additional research has provided supporting evidence that there are age-related differences to nicotine sensitization in adolescent and adult models. Between-group comparison evaluates separate samples of participants placed within the same treatment conditions. When this research design is implemented in animal drug studies, it reveals that, following acute nicotine exposure, adolescent animals spend increased time in the arms of the elevated plus maze. However, under the same conditions, their adult counterparts spend less time in the arms of the elevated plus maze (Elliot, Faraday, Phillips, & Grunberg, 2004; Kupferschmidt, Funk, Erb, & Le, 2010). Increased time spent within the open arms of the elevated plus maze suggests decreased levels of anxiety and increased positive reward in the affected group. This furthers the argument that adolescence is a susceptible time period for the effects of drug experimentation.

Exploratory behavior is one measure of anxiety-like behavior in animal models commonly used for testing the effects of anxiolytics, drugs typically used to reduce or prevent anxiety attacks in adult humans (Blokland & Raaijmakers, 1993; Boguszewski & Zagrodzka, 2002; Li, Watanabe, Fujisawa, & Shibuya, 1995; Miyagawa, Hasegawa, Fukuta, Amano, Yamada, & Nabeshima, 1998). It has been found that there are age-dependent changes in emotional reactivity when animals are exposed to the open field

and hole box tests (Boguszewski & Zagrodzka, 2002; Blokland & Raaijmakers, 1993; Li et al., 1995; Miyagawa et al., 1998); specifically, there are increased levels of anxiety in both adult mice (Boguszewski & Zagrodzka, 2002; Lamberty & Gower, 1993) and rats (Boguszewski & Zagrodzka, 2002; Frussa-Filho, Otoboni, Giannotti, Amaral, & Concericao, 1992).While research has taken an in-depth look at the effects of anxiolytics in adult animals, few studies have looked at baseline anxiety levels of animals, beginning in adolescence and leading into adulthood (Smith & Morrell, 2007; Spear, 2000).

The elevated plus maze is one of the most commonly used models of animal anxiety, specifically in pharmacological experiments. The apparatus consists of a plus-shaped shelf with two open and two enclosed arms, each with an open roof and raised from the floor. The plus maze task is based on the theory that rodents harbor a natural aversion to open space with a conflicting drive to explore a novel environment. Aversion to the environment leads to confining movement within the enclosed arms, while anxiety reduction is indicated by increased time spent in the open arms. Both forced and voluntary movement into the open arms of the elevated plus maze are correlated with elevated plasma corticosterone levels, increased freezing, and production of fecal boli (Hogg, 1996; Pellow, Chopin, File, & Briley, 1985), and are representative of increased anxiety-like behavior in the animal (Hogg, 1996). While some studies have found increased anxiety-like behavior in adult rats (Frussa-Filho et al., 1992), these findings are inconsistent across current research.

There have also been conflicting findings on the concept of age-related motor decline. While some studies suggest there is, in fact, a decline in motor movement (Gage,

Dunnett, & Bjorkland, 1984; Ingram, London, & Goodrick, 1981; Lamberty & Gower, 1993), others disagree with this notion, indicating that no such motor decline is present with increasing age (Hofecker, Kment, Niedermuller, & Said, 1974; Janicke, Schulze, & Coper, 1983; Marshall, 1982). Research conducted by Boguszewski and Zagrodzka (2002) using the elevated plus maze and open field behavior modules has shown that anxiety and motor activity are independent from each other in both young and old age groups, showing that anxiety-like behavior present in older rats is not the result of a decreased level of motor activity.

Development of neurobiological systems and emotions continues throughout adolescence and into adulthood. This can affect risk-taking behaviors and decision-making during this time period, which can lead to drug experimentation. It is important to continue research on the development of anxiety in order to understand how emotional dynamics can provide insight into the development of addiction. While there is a difference between adults and adolescents in neurobiological and emotional development, it is still unknown to what degree these mechanisms play a role in drug addiction. Further information is needed about the development and cycle of anxiety throughout adolescence and into adulthood to further understand the effects on drug addiction, withdrawal, and relapse. Through investigation of baselines of anxiety-like behavior through adolescence into adulthood, it will be possible to provide insight into how emotional development and anxiety correspond with drug addiction at different ages throughout this time period. Specific age ranges that are especially vulnerable to drug addiction during emotional development will also be examined.

The present experiment investigated age-related changes in anxiety-like behavior in a rat population, using separate cohorts of animals to specifically examine seven time periods throughout the course of adolescence into adulthood. The age cohorts were then grouped to represent early adolescence, mid-adolescence, late adolescence, and adulthood. This experiment was conducted through the use of the elevated plus maze and open field apparatus models. The elevated plus maze examined the time spent in the open arms of the elevated plus maze, number of entries into the open arms of the maze as a function of age, and latency to enter the open arms of the apparatus as a function of anxiety. The total distance of open-field locomotor activity was also measured as an index of anxiety-like behavior. These data provide preliminary evidence for the role of age-related changes in emotional processing that may reduce the rates of substance abuse, dependence and withdraw.

Behavioral models of anxiety of four separate groups of animals at different age cohorts were analyzed and compared on multiple measures. It was hypothesized that there would be age-specific differences in anxiety levels between groups throughout the adolescent and adult time period. Specifically, it was hypothesized that the age groups corresponding with mid-adolescence would have decreased levels of anxiety-like behavior when compared with pre-adolescent and adult groups. It was also hypothesized that these data will correlate with existing nicotine conditioned place preference and addiction data, showing that those animals in the mid-adolescent time frame form stronger preferences to nicotine than that of their adult counterparts. The results of these

hypotheses will examine the notion that adolescence is a vulnerable time period for the development of anxiety-like behavior and possible drug experimentation and addiction.

METHODS

Participants

Eighty (*N* = 80) male Sprague-Dawley rats were obtained from Harlan/Sprague-Dawley. The colony's temperature and humidity were monitored with an average of ~70 degrees, and ~40% humidity. The rats in the colony were maintained on a 12-hour light/dark cycle (0700-1900hrs) with access to food (Harlan-teklab #7012) and water *ad libitum*. All rats were group housed in plastic cages (approximately four animals per cage). Rats were housed according to assigned participation to one of the following age cohorts p28, p32, p36, p40, p44, p48, or p80 and were not handled except during cleaning and feeding procedures. Different groups of animals were used in each age category and were assigned as follows: early adolescence (p28, p32) = 20 males; mid adolescence (p36-p40) = 30 males; late adolescence (p44,p48) = 20 males; adulthood (p80)= 10 males. All treatment and housing procedures followed federal animal care and use guidelines in accordance with George Mason University guidelines and National Institute of Health Guide for the Care and Use of Laboratory Animals

Apparatus

After a one-week adjustment period (at post-natal p28 for the first group of animals), the first cohort of animals was tested in both the open field apparatus and the

elevated plus maze apparatus. The rats were tested first in the open field chamber and then in the elevated plus maze to assess the levels of anxiety present. Subsequent cohorts of different animals at each age group were tested at p32, p36, p40, p44, p48, and p80. The open field test consisted of a novel large area containing an aversive central area. The elevated plus maze is based on the conflict that rodents have between the drive to explore new environments while simultaneously fearing elevated open areas.

Open Field Apparatus

The open field apparatus consisted of a large arena made of wood, covered with white laminate, and surrounded by white walls that are 40 cm high. The floor of 100 cm × 100 cm was divided by black lines into 25 squares of 20 cm × 20 cm. The illumination in the test room was controlled to provide seven lux inside the apparatus. Each rat was placed in the center of the open field, and its behavior was registered for 15 minutes, with four rats tested at a time. Total distance traveled within the open field was observed as the dependant variable. After 15 minutes of testing, the animals were replaced in their home cage in the testing room until open field testing was complete. After termination of each testing session, the apparatus was cleaned with a water and alcohol solution. At the culmination of testing, rats were returned to their colony for 45 minutes until the session of elevated plus maze testing began. Testing in separate age cohorts occurred at p28, p32, p36, p40, p44, p48, and p80.

Elevated Plus Maze

The elevated plus maze was made of wood covered with black laminate and consisted of a plus-shaped platform elevated 52 cm from the floor. Two opposite arms (50 cm × 10 cm) were enclosed by 40 cm high walls, whereas the other two arms had no walls, only a surrounding ledge (1mm thick and 5mm high) to keep rats from falling off the arms. The four arms had at their intersection a central platform (10 cm×13.5 cm), which provided access to any of the four arms. The illumination at the ends of the open arms was between two to three lux. At the beginning of each test, the rat was placed in the central platform facing an alternating open arm. Behaviors were monitored for five minutes via a video camera mounted above the apparatus and watched on a TV screen by an experimenter located in the adjacent room. The dependant variables observed were: the number of entries and the time spent (with all four paws) in each type of arm; the latency to enter the open arms; and the total number of arm entries. The apparatus was cleaned after each individual testing session with the same water and alcohol solution used in the open field testing apparatus.

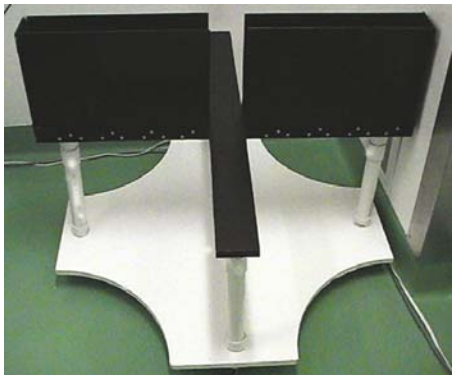

Figure 1: Elevated Plus Maze (Walf & Frye, 2007).

Viewpoint Tracking

Viewpoint automated tracking system was used to monitor, track, and quantify locomotor activity. The viewpoint system detected the contrast between the animal and the background of the testing chamber. Once the animal was located by the system, the viewpoint console quantified the animal's axial or retrograde locomotor activity. The viewpoint console was used to calculate total distance traveled in centimeters by each animal within the open field apparatus. Viewpoint also provided digital video files that were scored for elevated plus maze behavior.

Statistical Analyses

One way analysis of variance (ANOVA) between groups was conducted on four separate age groups in early adolescence, mid-adolescence, late adolescence, and adulthood (p28-p32, p36-p40, p44-p48, and p80) was used to determine the significance of anxiety in adolescence compared with adulthood. The ANOVA was designed with independent variables set as follows; age of the animal (p28-p32, p36-p40, p44-p48, and p80). The dependent variable (DV) was set as the distance traveled in centimeters in open field (DISTANCE), percent entry into open arms (PERCENT ENTRY), the latency to enter the open arms (LATENCY TO ENTER), and the percent time spent in the open arms (PERCENT TIME).

RESULTS

Open Field Apparatus

Total distance traveled. There was a significant main effect of age ($F_{1,3}=4.23$, $p=.008$) (See Figure 1). Specifically, a Tukey HSD post hoc analysis indicates that there was a significant difference between the animals in the p44-p48 age cohort and animals in the age cohort p28-p32 ($F_{1,3}=4.23$, $p=.004$). The results show total distance traveled in the open field apparatus gradually increasing until reaching the peak activity level at p44-p48 and sharply decreasing at the p80 age cohort. This suggests that rats in the p44-p48 age cohort showed increased locomotion overall corresponding with decreased anxiety, more specifically when compared to the animals in the age cohort p28-p32.

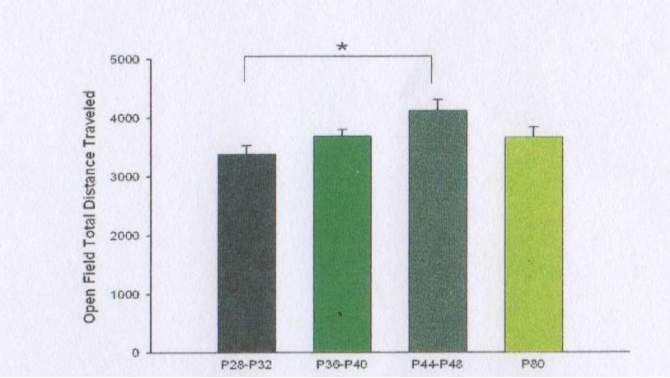

Figure 2: Total distance traveled in the open field apparatus.

Elevated Plus Maze

Percentage of time on open arms. There was a significant main effect of age on the percentage of time spent in the open arms of the elevated plus maze, *($F_{1,3}$=6.36, p=.001)* (See Figure 2). Tukey HSD post hoc analysis indicates that the p28-p32 cohort is significantly different from the p36-p40 cohort (*$F_{1,3}$=6.36, p=.002)* as well as from the p44-p48 cohort (*$F_{1,3}$=6.36, p=.002).* These results suggest that the percentage of time spent in the open arms is a function of age, with increased anxiety-like behavior in the early adolescent age cohort (p28-p32).

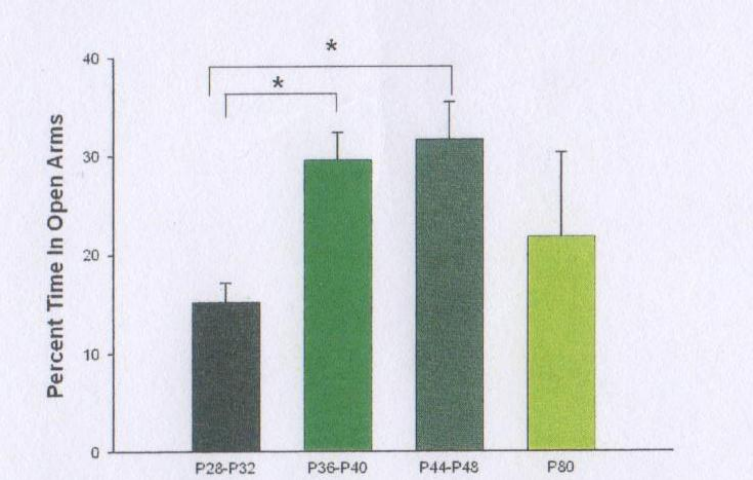

Figure 3: Percent time spent in the open arms of the elevated plus maze.

Percentage of open arm entries. There was a significant main effect of age on the percentage of entries into the open arms of the elevated plus maze, (*$F_{1,3}$=4.45, p=.006)* (See Figure 3) . Tukey HSD pos hoc analysis indicates that the p28-p32 cohort is significantly different from the p36-p40 cohort (*$F_{1,3}$=4.45, p=.05)* as well as from the p44-p48 cohort (*$F_{1,3}$=4.45, p=.021).* These results suggest that the percentage of entries

into the open arms is a function of age, with increased anxiety-like behavior in the early adolescent age cohort.

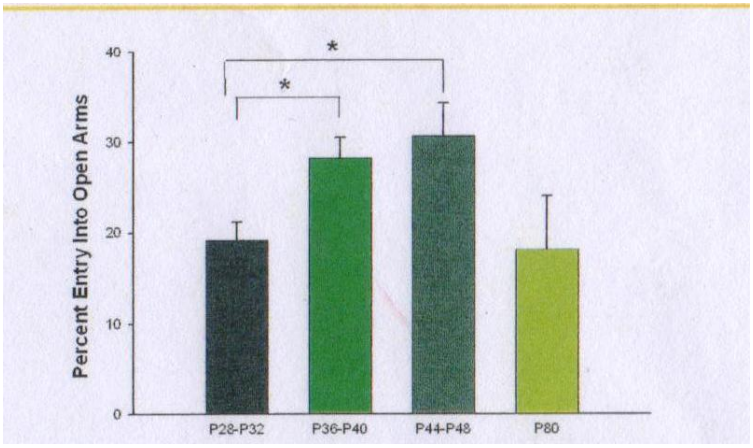

Figure 4: Percent entry into open arms of the elevated plus maze.

Latency to enter the open arms. There was a significant main effect of age on the animals latency to enter the open arms of the elevated plus maze ($F_{1,3}=2.89$, $p=.040$). While specific age cohorts did not reach significance when compared with each other, those animals in the p28-32 cohort and the p80 cohort exhibit an increased latency to enter the open arms of the elevated plus maze. These data suggest that there is an overall latency to enter the open arms of the elevated plus maze that varies with age, exhibiting increased anxiety-like behavior in the early adolescent as well as the adult age cohort.

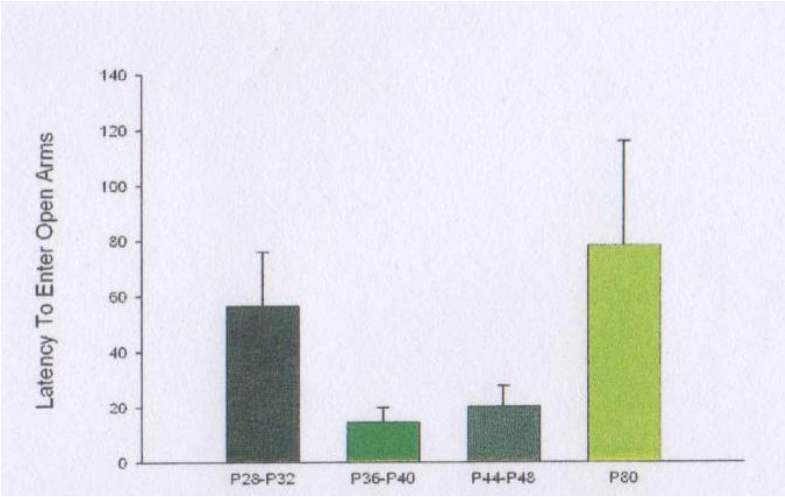

Figure 5: Latency to enter the open arms of the elevated plus maze.

DISCUSSION

Adolescence is a time of major brain growth and development, which underscores the importance of investigating emotional development of anxiety during this time period. While little research has examined behavioral development during adolescence in animal models, the current study aimed to determine the development of anxiety-like behavior in rats throughout adolescence into adulthood. The results indicated that anxiety-like behavior is partly a function of age, with early adolescent and adult animals exhibiting higher levels of anxiety-like behavior than that of their mid- and late adolescent counterparts. More specifically, animals in the p28-p32 age cohort, when compared with the p36-p40 and p44-p48 age cohort, exhibited increased anxiety-like behavior in total distance traveled in the open field apparatus, percentage of time spent on the open arms of the elevated plus maze, as well as the percentage of entries into the open arms of the elevated plus maze. The p28-p32 and the p80 age cohort also exhibited increased anxiety-like behavior with latency to enter the open arms of the elevated plus maze.

These results conflict with emotional development studies exhibiting that anxiety levels decreased in a linear fashion from adolescence into adulthood (Lynn & Brown, 2010). One potential methodological difference is the lack of clear definition of age cohorts outlining the time periods of early adolescence, mid-adolescence, late adolescence, and adulthood across literature. While some researchers mark adolescence as postnatal days (p34-p39), late adolescence (p51-p55), young adulthood (p65-p69) and

older adulthood as (p104-p109) (Lynn & Brown, 2010), others suggest that adolescence begins earlier during early adolescence at, P27-P30; late adolescence, p38-p41, and adult p90-p99 (Belluzzi et al., 2004), thus, making it difficult to compare results for specific age groups without concrete time periods of development. There has also been conflicting literature on age-related motor decline; however, recent literature conducted in the elevated plus maze and open field models demonstrates that anxiety present in older rats is not the result of a decreased level of motor activity (Boguszewski & Zagrodzka, 2002). In addition to the methodological limitations that age may have played on this study, sex differences in anxiety between male and female rats were also not explored.

However, these results correspond with emotional development studies, which suggest that adolescent rodents show increased levels of exploration and novelty-seeking and, therefore, exhibit lower levels of anxiety-like behavior than adults (Spear, 2007). The results from the current study also correspond with addiction models of conditioned place preference, showing that adolescence is a critical period of nicotine initiation and continued use, more specifically; there are significant differences in nicotine sensitivity between early and late phases of adolescence (Belluzzi et al., 2004). The correspondence of these data suggests that emotional development changes may play a role in the differentiation between age cohorts responsiveness to drugs (Belluzzi et al., 2004).

The neurobiological mechanisms underlying age differences in anxiety are unclear. Adolescence, in particular, is a time of major brain growth and development including meoscorticolimbic dopmaine systems. The mesocorticolimbic (DA) system, which is involved in the regulation of emotions and stress can influence cross sensitization with drugs of abuse (Pani, Porcella, & Gessa, 2000). Expression of striatal dopamine receptors, the DA transporter and presynaptic dopaminergic markers are also at peak levels during adolescence (Brielmaier, McDonald, & Smith, 2007). Further research has exhibited that $GABA_BR1$ antibody expression reaches adult levels during adolescence in rats (Lopez- Bendito, Shigemoto, Kulik, Paulsen, Fairen, & Lujan, 2002). GABA is one of several neurotransmitters that appear to be involved in the pathogenesis of anxiety and mood disorders. These responses of brain development may reflect on age specific differences in anxiety during adolescence. Further information is needed about the development and cycle of anxiety throughout adolescence and into adulthood to further understand the effects on drug addiction, withdrawal, and relapse.

.

CONCLUSION

It is important to understand the development of anxiety in order to understand how emotional dynamics can provide insight into the development of addiction. The results of this study may have important implications for future addiction studies due to the fact that the development of neurobiological systems and emotions continues throughout adolescence and into adulthood. This can affect risk-taking behaviors and decision-making during this time period, which can lead to drug experimentation. Therefore, this model can provide significant preliminary information about the susceptibility of the development of anxiety by showing that developmental changes in the brain contribute to age-specific anxiety behaviors. These age-related developmental changes, sex differences between male and female rats and neurobiological developmental changes should be examined in conjunction in future drug model studies to understand how these changes may make adolescents susceptible to the use and abuse of alcohol and other drugs .

REFERENCES

REFERENCES

Anderson, S. L. (2003). Trajectories of brain development: Point of vulnerability or window of opportunity? *Neuroscience and Biobehavioral Reviews, 27*, 723-732.

Belluzzi, J. D., Lee, A. G., Oliff, H. S., & Leslie, F. M. (2004). Age-dependent effects of nicotine on locomotor activity and conditioned place preference in rats. *Psychopharmacology, 174*(3), 389-395.

Blokland, A., & Raaijmakers, W. (1993). Age-related changes in correlation between behavioral and biochemical parameters in Lewis rats. *Behavior Neural Biology, 60*, 52-61.

Boguszewski, P., & Zagrodzka, J. (2002). Emotional changes related to age in rats-A behavioral analysis. *Behavioral Brain Research, 133*, 323-332.

Breese, G. R., Chu, K., Dayas, C. V., Funk, D., Knapp, D. J., Koob, G. F., Le, D. A., O'Dell, L. E., Overstreet, D. H., Roberts, A. J., Sinha, R., Valdez, G. R., & Weiss, F. (2005). Stress enhancement of craving during sobriety: A risk for relapse. *Alcohol Clinical Experimental Research, 29*, 185-195.

Brielmaier, J. M., McDonald, C. G., & Smith, R. F. (2007). Immediate and long-term behavioral effects of a single nicotine injection in adolescent and adult rats. *Neurotoxicology and Teratology*, 74-80.

Brown, S. A., & Tapert, S. F. (2004). Adolescence and the trajectory of alcohol use: Basic to clinical studies. In R. E. Dahl & L. P. Spear (Eds.), *Adolescent brain development: Vulnerabilities and opportunities* (pp. 234-244). New York, NY: New York Academy of Sciences Press.

Buckner, J. D., Timpano, K. R., Zvolensky, M. J., Sachs-Ericsson, N., & Schmidt, N. B. (2008). Implications of comorbid alcohol dependence among individuals with social anxiety disorder. *Depression and Anxiety, 25*, 1028-1037.

Chiang, S. S., Schuetz, C., & Soyka, M. (2005). Effects of cue exposure on the subjective perception of alcohol dependents with different types of cue reactivity. *Journal of Neural Transmission, 112,* 1275-1278.

Cooney, N. L., Litt, M. D., Morse, P. A., Bauer, L. O., & Gaupp, L. (1997). Alcohol cue reactivity, negative-mood reactivity, and relapse in treated alcoholic men. *Journal of Abnormal Psychology, 106,* 243-250.

Dahl, R. E., & Spear, L. P. (2004). Adolescent brain development and animal models. In R. E. Dahl & L. P. Spear (Eds.), *Adolescent brain development: Vulnerabilities and opportunities* (pp. 23-26). New York, NY: New York Academy of Sciences Press.

Elliot, B. M., Faraday, M. M., Phillps, J. M., & Grunberg, N. E. (2004). Effects of nicotine on elevated plus maze and locomotor activity in the male and female adolescent and adult rat. *Pharmacological Biochemistry and Behavior, 77,* 21-28.

Feldstein Ewing, S. W., Filbey, F. M., Chandler, L. D., & Hutchinson, K. E. (2010). Exploring the relationship between depressive and anxiety symptoms and neuronal response to alcohol cues. *Alcoholism: Clinical and Experimental Research, 34*(3), 396-403.

Fox, H. C., Berquist, K. L., Hong, K., & Sinha, R. (2007). Stress-induced and alcohol cue-induced craving in recently abstinent alcohol-dependent patients. *Alcohol Clinical Experimental Research, 31,* 395-403.

Frussa-Filho, R., Otoboni, J. R., Giannotti, A. D., Amaral, A. C., & Conceicao, I. M . (1992). Effect of age on antinociceptive effects of elevated plus-maze exposure. *Brazilian Journal of Biological Research, 25*(8), 827-829.

Gage, F. H., Dunnett, S. B., & Bjorkland, A. (1984). Spatial learning and motor deficits in aged rats. *Neurobiology of Aging, 5*(1), 43-48.

Grotheus, J. M., Bischof, G., Reinhardt, S., Meyer, C., John, U., & Rumpf, H.-J. (2008). Effectiveness of brief alcohol interventions for general practice patients with problematic drinking behavior and comorbid anxiety or depressive disorders. *Drug Alcohol Dependence, 94,* 214-220.

Hofecker, G., Kment, A., Niedermuller, H., & Said, H. (1974). Assessment of activity patterns of one- and two-year-old rats by electronic recording. *Experimental Gerontology, 9*(3), 109-114.

Hogg, S. (1996). Review of the validity and variability of the elevated plus-maze as an animal model of anxiety. *Pharmacology Biochemistry and Behavior, 54*(1), 21-30.

Ingram, D. K., London, E. D., & Goodrick, C. L. (1981). Age and neurochemical correlates of radial maze performance in rats. *Neurobiology of Aging, 2*(1), 41-47.

Janicke, B., Schulze, G., & Coper, H. (1983). Motor performance achievements in rats of different ages. *Experimental Gerontology, 18*(5), 393-407.

Johnston, L. D., O'Malley, P. M., Bachman, J. G., & Schulenberg, J. E. (2005). *Monitoring the Future national survey results on drug use, 1975-2010. Volume I: Secondary school students*. Ann Arbor, MI: Institute for Social Research, The University of Michigan.

Kupferschmidt, D. A., Funk, D., Erb, S., & Le, A. D. (2010). Age-related effects of acute nicotine on behavioural and neuronal measures of anxiety. *Behavioral Brain Research, 213,* 288-292.

Kushner, M. G., Abrams, K., Thuras, P., Hanson, K. L., Brekke, M., & Sletten, S. (2005) Follow-up study of anxiety disorder and alcohol dependence in comorbid alcoholism treatment patients. *Alcohol Clinical and Experimental Research, 29*, 1432-1443.

Lamberty, Y., & Gower, A. J . (1993). Spatial processing and emotionality in aged NMRI mice: a multivariate analysis. *Physiological Behavior, 54*(2), 339-343.

Li, J. W., Watanabe, M., Fujiwasa, Y., & Shibuya, T. (1995). Relation between age-related changes in hyper-emotionality and serotonergic neuronal activities in the rat limbic system. *Nihon Shinkei Seishin Yakurigaku Zasshi, 15*(3), 231-238.

Litt, M. D., Cooney, N. L., & Morse, P. (2000). Reactivity to alcohol-related stimuli in the laboratory and in the field: Predictors of craving in treated alcoholics. *Addiction, 95*, 889-900.

Lopez- Bendito, G., Shigemoto, R., Kulik, A., Paulsen, O., Fairen, A., & Lujan, R. (2002). Expression and distribution of metabotropic GABA receptor subtypes GABABR1 and GABABR2 during rat neocortical development. *European Journal of Neuroscience*, *15*, 1766-1778.

Lynn, D. A., & Brown, G. R. (2010). The ontogeny of anxiety-like behavior in rats from adolescence to adulthood. *Developmental Psychobiology*, 52(8), 731-739. DOI: 10.1002/dev.20468.

Marshall, J. F. (1982). Sensorimotor disturbances in the aging rodent. *Journal of Gerontology, 37*(5), 548-554.

Miyagawa, H., Hasegawa, M., Fukuta, T., Amano, M., Yamada, K., & Nabeshima, T. (1998). Dissociation of impairment between spatial memory, and motor function and emotional behavior in aged rats. *Behavior Brain Research, 91*(1-2), 73-81.

Pani, L., Porcella, A., & Gessa, G. L. (2000). The role of stress in the pathophysiology of the dopaminergic system. *Molecular Psychiatry*, 5, 14-21.

Pellow, S., Chopin, P., File, S. E., & Briley, M. (1985). Valdation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *Journal of Neuroscience Methods, 12*, 149-167.

Romeo, R. D. (2005). Neuroendocrine and behavioral development during puberty: A tale of two axes. *Vitamins and Hormones, 71*, 1-25.

Sinah, R. (2007). The role of stress in addiction relapse. *Current Psychiatry Reports, 9*, 388-395.

Sinah, R., Fox, H. C., Hong, K. A., Berquist, K., Bhagwagar, Z., & Siedlarz, K. (2008). Enhanced negative emotion and alcohol craving, and altered physiological responses following stress and cue exposure in alcohol dependent individuals. *Neuropsychopharmacology, 34*, 1198-1208.

Smith, K. S., & Morrell, J. I. (2007). Comparison of infant and adult rats in exploratory activity, diurnal patterns, and responses to novel and anxiety-provoking environments. *Behavioral Neuroscience, 121*(3), 449-461.

Smith, R. F. (2003). Animal models of periadolescent substance abuse. *Neurotoxicology and Teratology, 25*(3), 291-301.

Spear, L. P. (2000). Neurobehavioral changes in adolescence. *Current Directions in Psychological Science, 9*(4), 111-114.

Turner, L., Mermelstein, R., & Flay, B. (2004). Individual and contextual influences on adolescent smoking. In R. E. Dahl & L. P. Spear (Eds.), *Adolescent brain development: Vulnerabilities and opportunities* (pp. 175-197). New York, NY: New York Academy of Sciences Press.

Walf, A., Frye, C. (2007). The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nature Protocols, 2*, 322-328.

Weinerger, A. H., Desai, R. A., & McKee, S. A. (2010). Nicotine withdraw in U.S smokers with current mood, anxiety, alcohol use, and substance use disorders. *Drug and Alcohol Dependence, 108*, 7-12.

CURRICULUM VITAE

Shealyn G. Holt received her Bachelor of Arts in Psychology from Villanova University in 2005. She went on to enroll in the Master of Arts in Biopsychology at George Mason University in 2006. While enrolled in her Masters of Arts program, she began a position as the National Research Coordinator for the Defense and Veterans Brain Injury Center. She has since been promoted and currently is employed as the Family Caregiver Program Manager for the Defense and Veterans Brain Injury Center.