Individual Variance in Locomotor Behavior and D2 mRNA After Acute Nicotine

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By

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# DEDICATION

This is dedicated to my parents, Patricia and Frank Pruss, and Nicholas R. Falco, Jr., and to my brother, Nicholas Raphael Falco, III.

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The research in this master's thesis has been financially supported by the Virginia Tobacco Settlement Foundation (VTSF).

I am grateful for the support, emotional and otherwise, that my friends, family, and other supporters supplied during this time. My friend and peer, Charles Blanchard, assisted me in numerous parts of research and helped me move along. I am also grateful to my committee, Drs. Smith, Bachus, and McDonald for their contributions, suggestions, and the expenditure of their valued time.

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LIST OF ABBREVIATIONS
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| Abbreviation | Definition |
|---|---|
| CPP | conditioned place preference |
| DA | dopamine |
| DAT | dopamine transporter |
| EPM | elevated plus maze |
| HR | high responder |
| LR | low responder |
| mPFC | medial prefrontal cortex |
| NAc | nucleus accumbens |
| OF | open field |
| 6-OHDA | 6-hydroxydopamine |

ABSTRACT

INDIVIDUAL VARIANCE IN D2 MRNA AFTER ACUTE NICOTINE

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Tobacco use is one of the leading health concerns in the world. Of particular concern is the unique vulnerability that adolescents exhibit for nicotine use and addiction which has been examined via animal models. This project examines the effects that a single injection of nicotine has on early adolescent, late adolescent, and adult Long-Evans male rats. Rats were initially tested in the elevated plus maze at P28, P45, and P80 as a screen for anxious behavior. The next day, all animals received a saline injection and were placed in the open field to determine baseline locomotor behaviors. On Day 3, all animals received a single injection of nicotine (0.4 mg/kg, s.c., freebase) and locomotor variables were again determined. Twenty-four hours after nicotine injection, rats were sacrificed and tissue targeting the nucleus accumbens was sliced using a cryostat. *In situ* hybridization was completed to analyze the expression of D2 mRNA in the core and shell of the nucleus accumbens. When all three age groups were compared to each other, adults in the elevated plus maze spent more time in the center than either early or late

adolescents. In the open field, early adolescents traveled more total distance than either late adolescents or adults and traveled more distance in the center than adults. Using the data from these two behavioral tests, simple regression equations could be calculated that were predictive of D2 mRNA expression in the core and shell among early adolescents and adults. Interestingly, elevated plus maze variables were more predictive of D2 expression in early adolescents and open field variables were more predictive of D2 expression in adults. This suggests that a rat's expression of D2 mRNA in the NAc core and shell may be related to differential behavioral variables at different age points.

# INTRODUCTION

Tobacco is well known to be associated with a multitude of health problems. Nicotine has long been thought to be the primary addictive component of tobacco, though numerous other factors, including the presence of flavorings, ammonia, monoamine oxidase inhibitors, and acetaldehyde, may increase the addictive potential of tobacco (Cao, Belluzi, Loughlin, Keyler, Pentel, & Leslie, 2007; Rose, 2006). Rates of dependence in the general population for nicotine have been shown as being higher than those for alcohol, marijuana, or cocaine, with a lifetime dependence of 24.1% in the US population between the ages 15 and 54 (Anthony, Warner, & Kessler, 1994).

## Individual Differences

One question that has always arisen in addiction research is that of "What makes one person an addict over another?" This topic is partially researched by addressing individual differences or individual variability—the factors that may control addictive potential. These factors include genetic factors, including ethnic or gender differences, neurotransmitter variations, and behavioral factors, such as early environment, stressors, or chronic drug consumption (Brody, et al., 2009; Deminière, Piazza, Le Moal, & Simon, 1989; Perkins, 1995). It has also been observed that the craving that is experienced after a single intake differs from individual to individual (Deminiere, et al., 1989). Age of

initiation of drug use has also been discerned as playing a role in later addiction (Koob & Le Moal, 2008).

There are numerous animal models of individual differences in drug addiction that have been useful in examining various aspects of these properties. These models include self-administration, conditioned place preference (CPP), and locomotor response to a novel environment (Dadmarz & Vogel, 2003; Deminière, et al, 1989; Piazza, Deminière, Le Moal, & Simon, 1989; Rosecrans, 1995). Each of these animal models has been helpful in teasing apart the various factors that make up an individual's vulnerability to drugs of abuse, including nicotine. Locomotor response to a novel environment, both after an acute dose of nicotine and after repeated dosing, has often been used to examine individual differences and to predict later drug behaviors in animals. The research surrounding this model will be further examined here.

Age

Age of drug use onset is an aspect of individual variability that has been significantly related to later drug use. In a study of individuals who have smoked daily for one month or more, 33.6% had their first cigarette at age 13 or younger, 43.2% smoked their first cigarette at 14 to 16, and 23.2% started smoking at 17 or older, suggesting that the vast majority of smokers began use at age 16 or before (Breslau & Peterson, 1996). Those who began smoking at age 17 or older were also more likely to quit smoking than their younger counterparts. Later research also supported these findings, showing that the majority of smokers began smoking between the age of 14 and 17 (55%) and smoking in early adolescence was correlated with a lower likelihood of

quitting (Chen & Millar, 2000). Adolescent smokers are also more likely to report tolerance than adults (22.2% vs. 14.4%, respectively) and have higher rates of dependence than adults even though adolescents smoke fewer cigarettes a day than adults (Kandel & Chen, 2000). Animal models have also been useful in determining the impact of age on drugs of abuse (Spear, 2000). In animal models, it has been shown that adolescents have a higher vulnerability to the rewarding effects of nicotine (Adriani, et al, 2003; Torres, Tejeda, Natividad, & O'Dell, 2008).

Studies have shown various interactions between age and nicotine in animal models. Some studies find that nicotine inhibits locomotor behavior, or has no effect on locomotor behavior in adolescent rats, particularly during an acute dose. Belluzzi and colleagues found that a single dose of nicotine (0.125-0.5 mg/kg, s.c., freebase) inhibited locomotor activity in adult and late adolescent rats, but had no impact on early adolescents (Belluzzi, Lee, Oliff, & Leslie, 2004). In a study comparing male and female rats, male adolescent rats did not show any significant differences in locomotor behavior after nicotine (6 mg/kg/day, osmotic pumps) dosing (Trauth, Seidler, & Slotkin, 2000).

Other research indicates that acute doses of nicotine have stimulatory effects on locomotion. During acute nicotine dosing (0.4 mg/ml/kg, s.c.), adolescent rats showed increases in locomotor behavior while adult rats showed increases in locomotor behavior after repeated nicotine dosing (Schochet, Kelley, & Landry, 2004). Similar results have been exhibited by other labs at similar nicotine doses (Elliot, Faraday, Phillips, & Grunberg, 2004). Further research has indicated that adult rats are more active than adolescents after an acute nicotine (0.5, 1.0 mg/kg, s.c.) injection (Faraday, Elliott,

Phillips, & Grunberg, 2003). The higher activity levels in adult rats versus adolescent rats also continue after repeated nicotine (0.4 mg/kg, s.c.) injections and osmotic pump nicotine (12 mg/kg/day) dosing (Cruz, DeLucia, Planeta, 2005; Faraday, Elliott, & Grunberg, 2001).

It is of interest to determine why such contradictory results appear in this topic of the literature. It is possible that this can be explained by the ages that are used to describe the adolescent period. Various labs can describe adolescence as anywhere from postnatal day 28 (P28) to P44, and few labs break adolescence into early and late periods (Belluzzi, et al, 2004; Trauth, et al, 2000). Adolescence can clearly be a nebulous topic, with Spear (2000) generally defining periadolescence as P30-42 though some indications can appear as early as P28 and may last as late as P55 in male rats. It is possible that dividing adolescence into multiple periods may show variable locomotor results and would be beneficial to this model.

HR/LR Modeling

Little published research has examined the effects of injected or self-administered drugs on individual rats. Dadmarz and Vogel (2003) published a study suggesting that there was great variability in the individual amounts of nicotine solution self-administered in rats and that these values were capable of differing greatly from group means. A far more common methodology of statistical comparison is the high responder/low responder (HR/LR) model. In this model, rats are initially divided into those which have high initial levels of locomotor activity (HRs) and those which have low initial levels of locomotor activity (LRs) in a novel envirnoment (Rosecrans, 1995).

Across various models, nicotine tends to stimulate behavior in LRs and decrease behavior in HRs, though relationships can become varied and more complex.

The HR/LR model has been shown to be predictive of later CPP and self-administration models of cocaine and amphetamine. Some research has found that after cocaine dosing (10 mg/kg, i.p.), only LR rats alter their place preference ratios (Allen, Everett, Nelson, Gulley, & Zahniser, 2007). Other work has found no relationship between the HR/LR model and cocaine CPP (Gong, Neill, & Justice, 1996). HR/LR models were not found to predict the acquisition rates of cocaine (0.25 mg/kg/infusion) self-administration, but did seem to predict breakpoint during progressive ratio with HRs having a lower breakpoint (Carey, DePalma, & Damianopoulos, 2003; Mandt, Schenk, Zahniser, & Allen, 2008). However, selectively bred HR rats were found to acquire cocaine (0.2 mg/kg/50µl-poke) self-administration more quickly than LRs (Davis, Clinton, Akil, & Becker, 2008). Some research has also shown that HR/LR modeling is not predictive of amphetamine (1.5 mg/kg, i.p.) CPP behavior (Erb & Parker, 1994). Rats tested with amphetamine have shown that HRs are quicker to respond to the first amphetamine (1.5 mg/kg, i.p.) injection whereas LRs acquire amphetamine self-administration more quickly with repeated administration (Piazza, et al, 1989).

Initial separation into HRs and LRs has also been shown to be predictive of locomotor behavior when cocaine, amphetamine, or nicotine is administered and locomotor behavior is assessed in a novel environment. HRs have shown significantly greater locomotor activity after a cocaine (10 mg/kg, i.p.) injection (Allen, et al, 2007). This finding was supported in a study by Hooks and the findings were repeated with

amphetamine (0.5 mg/kg) (Hooks, Jones, Smith, Neill, & Justice, 1991a). Rosecrans (1971) found HRs to exhibit a reduction in locomotion while LRs exhibited an increase in locomotion after a nicotine (0.4 mg/kg, s.c.) injection. Kabbaj (2006) notes that the relationship between the HR/LR model and nicotine is complicated. If nicotine has a sedating effect on the cohort, studies show the relationship that Rosecrans discussed. If nicotine has a stimulatory effect overall, the HRs may appear to be less affected than LRs. According to Bevins and Besheer (2001), this may occur because the HRs start at a higher baseline level and seem to plateau whereas the activity levels of LRs are more pronounced.

The HR/LR model indicates that it is a promising method for analyzing individual variability in rats that are dosed with nicotine. To date, there has been relatively little research on this topic, though previous work in both nicotine and psychostimulants may give an idea of the potential for the model.

Nucleus Accumbens and Reward

Drugs of reward, including nicotine, tend to activate the same regions of the brain. One area that has been indicated in reward pathways is the nucleus accumbens (NAc). A multitude of evidence suggests that the locomotor stimulant effects of nicotine administration involve the mesolimbic system which includes the NAc (Balfour, Benwell, Birrell, Kelly, & Al-Aloul, 1998; Benwell, Balfour, & Khadra, 1994; Clarke, Fu, Jakubovic, & Fibiger, 1988; DiChiara, 2000; Imperato, Mulas, & DiChiara, 1986; Wise & Bozarth, 1987). The NAc is comprised of two components, the core and the shell, which appear to have different reactions to nicotine dosing (Carlezon & Thomas,

2009; DiChiara, 2000; Nisell, Marcus, Nomikos, & Svensson, 1997). Research suggests that the shell portion of the NAc is more sensitive to the stimulant and rewarding effects of drugs of abuse (Ikemoto, Glazier, Murphy, & McBride, 1997; Pontieri, Tanda, Orzi, & DiChiara, 1996; Sellings, Bahamouri, McQuade, & Clarke, 2008), though more recent research has indicated that both portions of the NAc are involved in the rewarding effects of nicotine, but differentially modulate the rewarding effects between D1-like receptors in the core and D2-like receptors in the shell (Laviolette, Lauron, Bishop, Sun, & Tan, 2008).

Dopamine and Nicotine Locomotor Responses

Dopamine is known to play a role in the locomotor response to nicotine. Benwell, Balfour, and Khadra (1994) found that repeated, but not acute, nicotine (0.25, 1.0, 4.0 mg/kg/day, infusion pumps) dosing increases extracellular concentrations of dopamine (DA) in the NAc. The same dose of nicotine also stimulated nicotine locomotion. However, previous research found that both acute and repeated nicotine (0.4 mg/kg, s.c., freebase) dosing increased extracellular DA concentrations in the NAc while stimulating locomotor behavior (Clarke et al., 1988; Benwell & Balfour, 1992). Evidence also exists indicating whether D1 or D2 receptors orchestrate the locomotor response to nicotine. After administration of SCH 23390 (0.001, 0.01, 0.1 mg/kg), a selective D1 antagonist, raclopride (0.05, 0.1 mg/kg), a selective D2 antagonist, fluphenazine (0.1, 0.3 mg/kg), a D1/D2 antagonist, SKF 38393, a selective D1 agonist, and PHNO (10µg/kg), a D2 agonist, it was found that all drugs except SKF 38393 had the expected impact on nicotine hyperlocomotion (O'Neill, Dourish, & Iversen, 1991). According to O'Neill et

al., these findings suggest that nicotine stimulates release of brain dopamine which excites both D1 and D2 receptors and causes hyperlocomotion. More recent research has investigated this topic, following similar protocol, though injections were directly into the NAc (Canales & Iverson, 2000). Similar to the O'Neill study, effects of SKF 38393 were modulated by co-administration of D2 and D3 agonists, suggesting that stimulation of D2 and D3 receptors may also play a role in causing hyperlocomotion.

The role that the NAc core and shell play in nicotine's locomotor response has also been differentiated. One study found that after an acute nicotine (25-100 µg/kg, i.v.) injection, increases occurred in extracellular DA in both the core and shell, with higher release in the shell (Nisell, Marcus, Nomikos, & Svensson, 1997). After a subsequent nicotine injection, the DA release was abolished in the core, but was still present, though to a reduced extent, in the shell. In another study, eticlopride, a DA antagonist, was injected into the core (0.0625-0.5 µg/side) and into the shell (0.5-1.0 µg/side) and 6-OHDA was infused to lesion the core or shell and the rats were then tested with a nicotine (0.2 mg/kg, s.c.) injection (Boye, Grant, & Clarke, 2001). However, Boye and colleagues found that the core, rather than the shell, contributed to the locomotor stimulant effects of nicotine.

There is evidence that dopamine levels can vary from animal to animal after nicotine administration and that HR/LR modeling can correlate with DA levels. In one study, after two injections of nicotine (0.4 mg/kg, s.c.), rats did not initially appear to show any changes from baseline extracellular DA levels in the NAc (Johnson, Zhao, James, & Rosecrans, 2000). However, a relationship formed when rats were analyzed

individually. If baseline DA levels were >5nM, administration of nicotine tended to decrease DA release. However, if DA levels were originally <5nM, nicotine injection tended to increase DA release. HR/LR status may also correlate with DA levels, but little work has been done on this topic, and none with nicotine. Rats that were classified as HRs based on locomotor response to a novel environment exhibited a greater dopamine response in the NAc after a cocaine (15.0 mg/kg, i.p.) injection (Hooks, Jones, Smith, Neill, & Justice, 1991b). HRs demonstrated a locomotor activity to DA ratio that was three times that of LRs. A study by Hooks et al. (1994) examined the DA profiles of both HR and LR rats. In the first experiment, DA was injected directly into the NAc (0, 3, 10, 30 µg/side) or the striatum (0, 10, 30, 100 µg/side). HR rats showed a greater locomotor response than LR rats to the 3 and 30 µg/side doses in the NAc. In the second experiment, radioligand binding assays were done to see if there were D1 dopamine receptor (D1DR) or D2DR binding site differences in NAc, striatum, or medial prefrontal cortex (mPFC). HR rats had fewer D2DR binding sites in the NAc and striatum than LR rats. There were no differences between HR and LR rats in D1DR binding sites in the striatum, or D1DR and D2DR binding sites in mPFC. Experiment three evaluated mRNA levels for D1 and D2 in NAc, striatum, and mPFC. HR rats had a decrease in D2 mRNA in the NAc compared to LR rats. There were no differences between HR and LR rats in either D2 or D1 for any of the other brain regions.

Finally, though not of relevance to this study, HR/LR modeling may be predictive of DAT levels. Briegleb et al. (2004) observed dopamine transporter (DAT) radioligand binding 30 minutes after cocaine (10 mg/kg, i.p.), amphetamine (0.5, 1 mg/kg, i.p.), or

saline injection in HRs and LRs. Cocaine HRs had significantly higher DAT uptake than LRs and locomotor activation correlated with DAT uptake. Animals injected with amphetamine did not show a difference in DAT uptake between HRs and LRs nor was there a correlation between locomotor activity and DAT uptake. Another study by Sabeti and colleagues (2003) investigated the relationship between cocaine HRs and LRs and DAT levels via electrochemical recording in the NAc. Rats were divided into HRs and LRs via locomotor activity in an open field arena and were assessed for DAT levels 30 minutes after receiving a cocaine (10 mg/kg, i.p.) or saline injection. HRs exhibited DAT inhibition, but LRs did not. These two studies suggest that the cocaine HR/LR model may be particularly useful, especially in predicting DAT levels. Overall, individual variability of DA levels and HR/LR modeling seem to have the potential to be an area of research worth further exploration.

Considering the growing amount of research on the differences between adolescents and adults in rodent models, there is surprisingly little research on the connection between age, dopamine and nicotine dosing. One interesting area of study has been fluctuations in DA levels in the brain. Studies have shown that DA levels peak in numerous areas of the rodent brain during adolescence. The densities of both D1 and D2 receptors peak and go through a marked decrease in the prefrontal cortex between P40 and P120, suggesting that the brain goes through pruning of D1 and D2 receptors (Andersen, Thompson, Rutstein, Hoststetter, & Teicher, 2000). However, this does not seem to occur in the NAc which experiences very slight reduction in D1 and D2 receptors during this time. In the NAc, D1 and D2 receptors appear to peak between P25 and P40,

decreased slightly around P60-80, but are the same at P 100-120 as at P40 (Teicher, Andersen, & Hostetter, 1995). These results are supported by a study done by Creese, Sibley, and Xu (1992) finding that striatal D1 and D2 mRNAs were only 50% of adult levels at birth, reached peak levels at P30, then decreased to final levels around P120. These data suggest that in the NAc, D1 and D2 fluctuate during adolescence to reach final levels around P100-120; this is an issue that cannot be ignored in attempting to measure dopamine receptors and performance between age groups.

In one study of interactions between age, dopamine and nicotine dosing, nicotine was administered by osmotic pump (6 mg/kg/day, freebase) at P30-P47.5 and control or nicotine animals received a dose of AMPT (300 mg/kg, i.p.), a tyrosine hydroxylase inhibitor, saline, or AMPT and nicotine (0.3 mg/kg, s.c.) in combination (Trauth, Seidler, Ali, & Slotkin, 2001). Dopamine levels were initially increased in the nicotine group during adolescent dosing, but reductions occurred post-treatment. Reductions in DA turnover were so significant that they continued until P80. Another study compared 7 days of nicotine (0.4 mg/kg, i.p.) dosing on adolescent (P30) and adult (P60) rats (Collins, Wade, Ledon, & Izenwasser, 2004). Autoradiographic receptor binding assays showed that neither D1 or D2 receptor densities in the NAc were different between nicotine animals and their controls in either adolescent or adult groups.

Due to the receptor pruning timing mentioned above, it is difficult to adequately compare age groups. However, there are two studies that have compared adolescent and adult age groups, though it seems that any changes in adolescents could be due to ontogenic differences in DA receptors. One study compared dopamine levels in the NAc

septi of adolescent (approximately P28) and adult (approximately P60-80) after nicotine (0.3 mg/kg, s.c.) injection (Shearman, Fallon, Sershen, & Lajtha, 2008). Animals that received nicotine injections had significantly higher levels in DA in the NAc than controls and adolescents had higher levels than adults. Another study examined the dopamine levels in the NAc of early adolescent (P35), late adolescent (P45), and adult (P60) rats that received four days of nicotine or saline injections (0.6 mg/kg, twice a day) and then acute or repeated nicotine (Badanich & Kirstein, 2004). Neither early nor late adolescents exhibited changes in dopamine levels due to nicotine exposure. However, adults that received acute nicotine showed increased DA levels in comparison to controls. In addition, DA levels were higher in adults than adolescents after acute nicotine injection. This finding is consistent with DA receptor research because if adolescents have more postsynaptic receptors, it is likely that DA levels will be lower as a compensatory response than in adults.

Conclusion

As can be seen, there is little specific research examining the effects of nicotine on DA in the NAc during varied age groups. Also of interest, no research has been published concerning this topic and individual variance in animal models unless the sparse research on HR/LR modeling is considered. The goals of this project are discussed below.

Specific Aims

1. To determine the effect of an acute, or single, dose of nicotine (0.4 mg/kg, s.c.) on the locomotor response to a novel environment and compare this behavior between an early adolescent, late adolescent, and adult group of male Long-Evans rats.

2. To determine if baseline HR/LR behavior is predictive of locomotor behavior after an acute injection of nicotine.

3. To determine DA D2 mRNA levels in the NAc core and shell via *in situ* hybridization.

4. To determine if DA D2 mRNA expression correlates with individual response to an acute nicotine injection, and if HR/LR modeling is predictive of DA D2 mRNA expression in the NAc.

# MATERIALS AND METHODS

## Subjects

Male Long-Evans hooded rats were obtained at postnatal day (P) 21, P38-39, and P73-74 (Harlan, Indianapolis, IN). Rats were group housed and maintained at constant temperature (22-24° C), on a 12 h L:D cycle (lights on at 7:00 am) with *ad libitum* food and water. Subjects were given an approximately seven day acclimation period to the colony prior to testing. Human handling was minimal with human exposure only during cage cleanings and feeding. Animal care was in accordance with George Mason University guidelines and National Institute of Health Guide for the Care and Use of Laboratory Animals.

## Elevated Plus Maze (EPM)

The experimental cohorts were comprised of an early adolescence group (P28, n = 20), a late adolescence group (P45, n = 20), and an adult group (P80, n = 20). On Day 1 of experimentation, all subjects were tested on the elevated plus maze (EPM) as a pre-anxiety screening. Testing lasted for 5 minutes and animals were scored on open arm time, open arm entries, and center time.

Locomotor Assessment and Drug Treatment

On Day 2 of the experiment, subjects were injected with saline and tested in an open field (OF) chamber (42 x 42x 30 cm) for 10 minutes to determine baseline locomotor levels. On Day 3, animals were injected with nicotine (0.4 mg/kg, s.c.) and assessed in the OF again to determine locomotor behavior. Locomotor behaviors were assessed on both the saline day and the nicotine day for center time and total distance traveled. Locomotor behaviors were assessed on total distance traveled, distance traveled in the center, and time spent in the center. Nicotine dose is expressed as freebase and all solutions were adjusted to pH 7.4 with NaOH.

High responder (HR) and low responder (LR) rats were identified based on their initial behavior in the OF chamber (based on total distance traveled) after saline injection. Response rates were again examined at the end of nicotine testing, again based on total distance traveled, to determine if initial HR/LR status was related to behavior after an acute injection of nicotine.

*In situ* hybridization

All rats tested in behavioral paradigms were used for *in situ* hybridization. Rats were sacrificed by guillotine decapitation 24 hours after the nicotine injection was given. Brains were quickly removed, frozen in powdered dry ice, and kept frozen in air-tight plastic freezer bags at –80° C until cryostat-sectioning. Tissue was cryostat cut in 16 µm coronal sections (Leica, Wetzlar, Germany) and thaw-mounted on a series of gelatin-subbed glass slides. Slides with NAc represented were selected from corresponding histological slides (stereotaxic atlas coordinates from 1.70 to 1.60 mm Bregma [Paxinos

& Watson, 1998]). The boundaries of the NAc were defined according to Paxinos and Watson (1998).

Oligonucleotide probes (Oligo's Etc., Wilsonville, OR) were radiolabeled, using terminal deoxynucleotidyl transferase and deoxyadenosine [alpha-[35S]-thio] triphosphate, at the 3' end, to a specific activity of 5-10 X $10^5$ cpm/µl, according to the method described by Young (1992).

Hybridization was carried out according to the method described by Young (1992). Briefly, warmed, dried sections were fixed in 4% formaldehyde/PBS for 5 minutes, rinsed in PBS, acetylated in 0.25% acetic anhydride/1 M triethanolamine hydrochloride (pH 8.0) for 10 min, dehydrated in graded ethanol, delipidated in chloroform for 5 min, rinsed in absolute and 95% ethanol, and air-dried. Hybridization buffer (50 µl) containing 50% formamide, 600 mM NaCl, 80 mM Tris-HCl (pH7.5), 4 mM EDTA, 0.1% sodium pyrophosphate, 0.2% sodium dodecyl sulfate, 0.2 mg/ml sodium heparin, 100mM dithiothreitol, 10% dextran sulfate, .01% cold polyadenylic acid, plus 1 X $10^6$ cpm of labelled probe, was then pipetted onto each slide, and parafilm coverslips added. Slides were incubated at 37° C overnight. Coverslips were then removed in SSC, and slides rinsed and collected in 1 X SSC, and washed in 4 changes of 1 X SSC at 60° C for 15 min each, and 2 changes of room temperature 1 X SSC for 30 min each. Slides were rinsed in water and 70% ethanol, and air-dried. All solutions used water treated with diethylpyrocarbonate (DEPC).

Probes

All probes used in *in situ* hybridization were 48-mer oligonucleotides. The rat D2 antisense sequence is 5' CTG CTG CGC TTG GTG TTG ACC CGC TTC CGG CGC TTC CGG AGG ACG ATG 3'. The control probe is a missense probe with the sequence 5' AAT ACA CCG AGC GGT ACT CGA GGT GGT ACA TGT TGG GGT AGT AAA TAA 3'.

Autoradiography and image measurement

Biomax film (Perkins Elmer, Boston, MA) was exposed to treated slides and $^{14}C$ standards (ARC Inc., St. Louis, MO) in a cassette, and then developed. Autoradiographic images of individual slides were converted to TIFF files with a flatbed scanner. Using NIH Image (Rasband, NIH), regions of interest were then sampled manually, with optical density interpolated along the calibration curve established from the standards.

Statistical Analyses

EPM data were analyzed initially using univariate ANOVAs for Age x Behavior for open arm time, open arm entries, and center time. Post hoc independent samples *t*-tests were used to differentiate between age groups when effects were found.

At the end of OF testing, total locomotor behavior after an acute dose of nicotine was determined by computing a difference score by subtracting Day 2 saline scores from Day 3 nicotine scores. Differences in total distance traveled, distance traveled in the center, and center time between age groups were determined via *t*-tests. To determine HR/LR status, a median split was conducted on animals within each age group based on their total distance traveled in the OF after saline injection. The top 50% of each age

group were considered to be HRs and the bottom 50% to be LRs. Independent samples *t*-tests were used to determine differences between HR and LR groups.

After D2 mRNA was sampled in the NAc and measured using the NIH Image program, mRNA levels in the core and shell were compared with both EPM and OF variables. Simple regressions were utilized in order to find mathematical equations relating behavioral variables with D2 mRNA levels in the NAc core and shell. Univariate ANOVAs using HR/LR status as a contrasting factor were also utilized to determine if HR/LR status was related to different levels of D2 mRNA in either the NAc core or shell.

# RESULTS

## EPM

There were no significant differences among age groups on either of the variables open arm time or entries into the open arm in the EPM (see Figures 1 and 2).

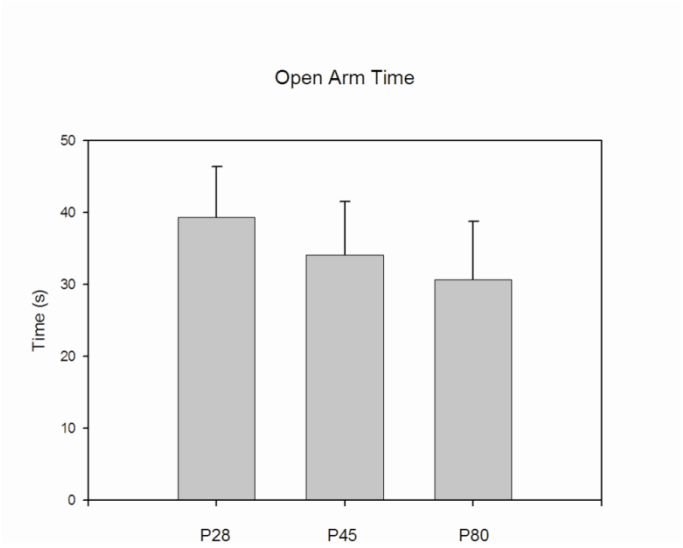


**Figure 1.** Open Arm time on the EPM in early adolescents (P28), late adolescents (P45), and adults (P80). There are no significant differences between age groups.

However, a univariate ANOVA showed a main effect for age for the variable of center time, $F(2,57) = 8.252$, $p \leq .001$. Independent samples *t*-tests were used to deconstruct the effects of age, and it was found that adults spent more time in the center of the EPM than either early adolescents, $t(38) = -3.573$, $p \leq .001$, or late adolescents $t(38) = -2.788$, $p < .01$ (see Figure 3).

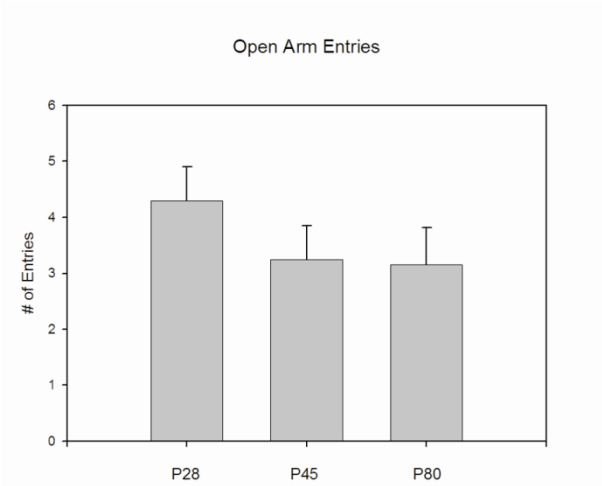


**Figure 2.** Open arm entries on the EPM in early adolescents (P28), late adolescents (P45), and adults (P80). There are no significant differences between age groups.

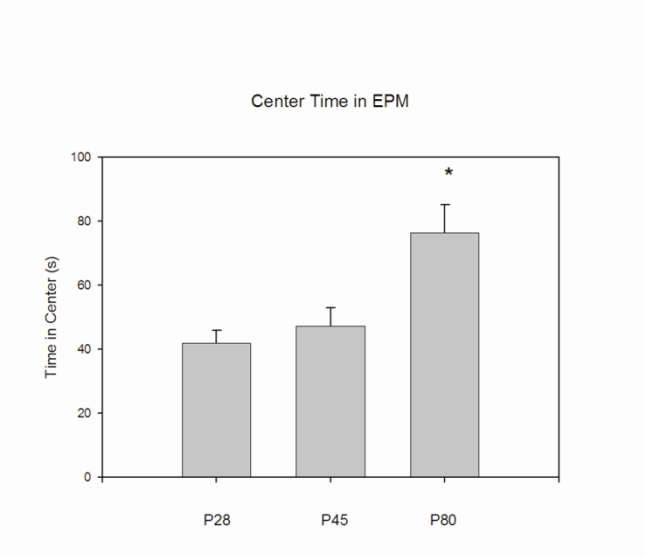


**Figure 3.** Time spent in the center on the EPM in early adolescents (P28), late adolescents (P45), and adults (P80). Adults spend significantly more time in the center than either early or late adolescents, $p < .05$.

Locomotor Assessment/Open Field

Locomotor behavior to an acute injection of nicotine was assessed in the OF. Two variables, total distance traveled and total distance traveled in the center, showed significant differences between age groups. A third variable, time spent in the center, did

not show any differences between age groups. On the total distance traveled variable, only the early adolescents (mean = 194.005) showed an increase in behavior after an acute injection of nicotine, while both late adolescents (mean = -372.005) and adults (mean = -622.860) showed decreases in behavior. There were significant differences between the early and late adolescent groups, $t(38) = 2.260$, $p < .05$, between the early adolescent and adult groups, $t(38) = 3.534$, $p \leq .001$, but not between the late adolescent and adult group (see Figure 4).

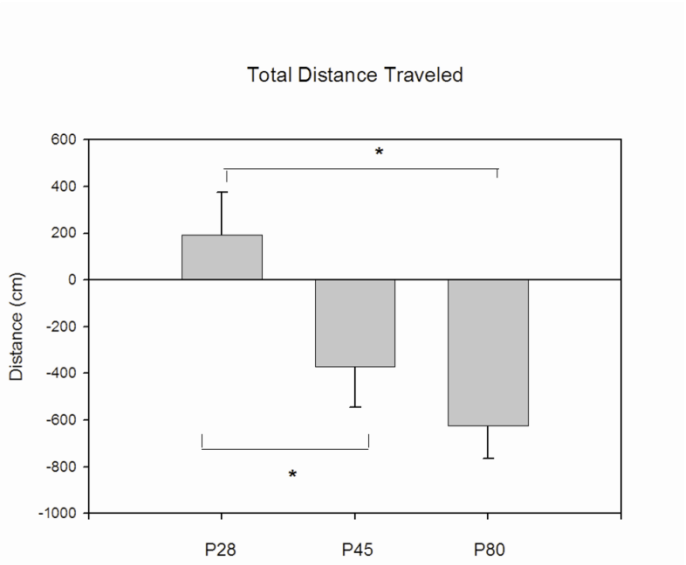


**Figure 4.** Total distance traveled in the open field. Significant differences are seen between early and late adolescents, and between early adolescents and adults, $p < .05$.

On the distance traveled in the center variable, significant differences were not seen between early and late adolescents, but did trend toward significance, $t(38) = 1.738$, $p < .10$. Significant differences were also seen between early adolescents and adults, $t(38) = 2.996$, $p < .05$, but not between late adolescents and adults (see Figure 5).

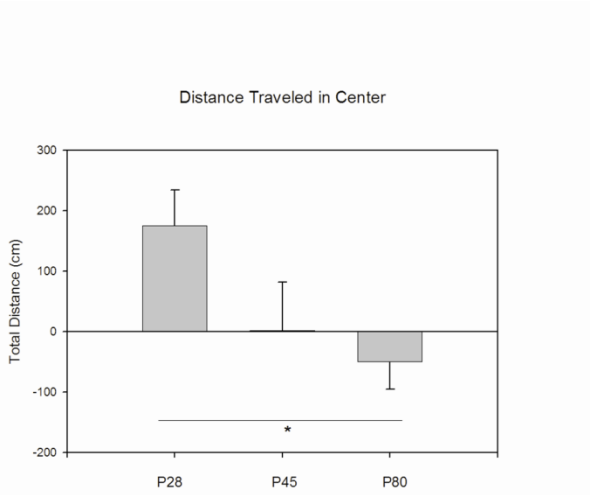


**Figure 5.** Total distance traveled in the center of the open field. Significant differences occurred between early adolescents and adults, $p < .05$. Differences between early and late adolescents trended toward significance, $p < .10$.

Use of the EPM as a pre-anxiety screening did not consistently predict performance in the OF. Performance in the EPM was analyzed for correlations on the measures of total distance traveled, total distance traveled in the center, and center time in the OF for all three age groups. The only significant correlation was between center time in the EPM and total distance traveled in the center of the OF for early adolescents, R = .468, $p < .05$ (Figure 6). Correlations between the two measures were non-significant for late adolescents, R = .273, $p = .24$ and adults, R = .153, $p = .52$.

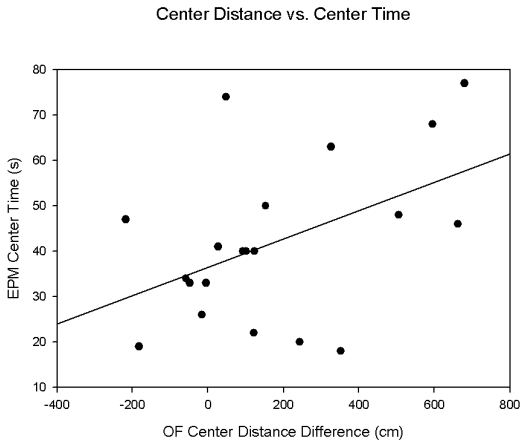


**Figure 6.** Correlation of total distance traveled in the center of the OF vs. center time in the EPM for early adolescents. R = .468, $p < .05$. This correlation was non-significant in late adolescents and adults.

## HR/LR Modeling

In order to select HRs and LRs, a median split was conducted on each group sampled. Only total distance traveled in the OF was assessed as this was most similar to previous work.

When all animals were condensed and treated as one sample, there were significant differences in total distance traveled between HRs and LRs, $t(58) = 2.626$, $p < .05$. However, when the ages were separated and HRs and LRs were compared within each group, only the HRs and LRs in the early adolescent group showed a significant difference, $t(18) = 3.129$, $p < .01$. HRs and LRs in the late adolescent and adult groups

showed no statistically significant difference (see Figure 7 for HR/LR data).

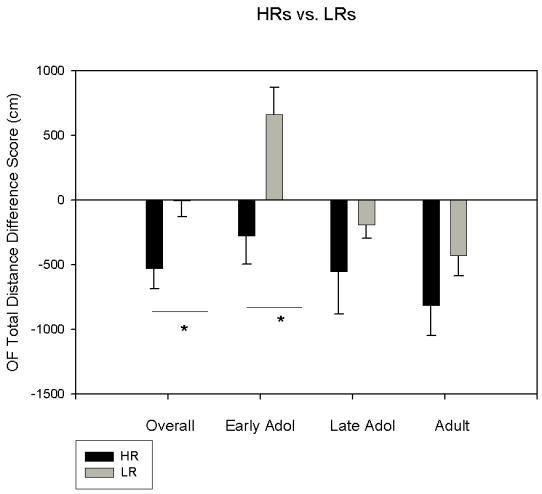


**Figure 7.** HR and LR performance on total distance traveled in the OF among the overall sample, early adolescents, late adolescents, and adults. Only significant differences between HRs and LRs appeared in the overall sample (when collapsed across ages) and in early adolescents, $p < .05$.

## *In Situ* Hybridization

Regional expression of D2 mRNA label relative to the control probe is shown in Figures 8 and 9. Across all animals, D2 mRNA expression was found to be significantly different in both the core and shell, $t(57) = 11.563$, $p < .001$, with expression being higher in the core.

Simple regressions were run between behavioral variables and either the core or shell D2 mRNA expression to determine if linear regression equations could be calculated relating behavioral variables and D2 expression. This was done across age groups within the entire subject pool, and then again within each age group. All equations will follow the general format:

core (or shell) = $b_0 + b_1$behavioral variable

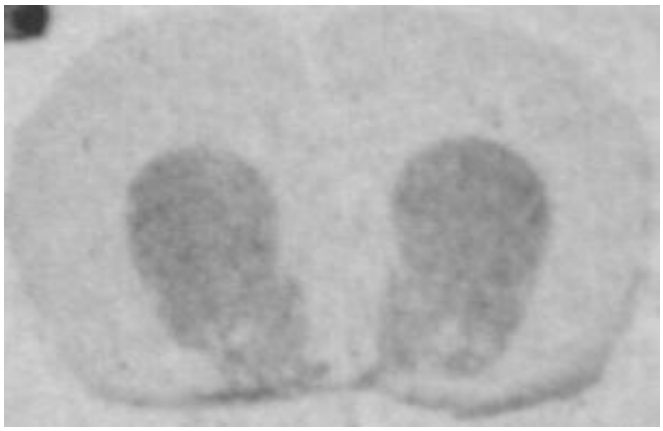

**Figure 8.** Representative image targeting nucleus accumbens that has been labeled with D2 antisense oligoprobe.

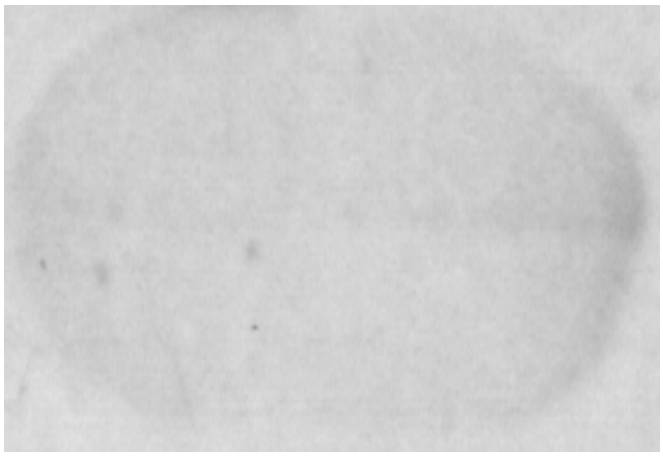

**Figure 9.** Representative image targeting nucleus accumbens that has been labeled with D2 missense oligoprobe.

These equations describe the best-fit line to each graph and give a method for relating independent and dependent variables.

No significant, or near significant results existed relating total distance traveled in the open field, which was thought to be a primary measurement of locomotor behavior, to D2 mRNA levels.

Univariate ANOVAs were conducted using HR/LR status as a contrasting factor. Again, statistics were run when the entire sample was collapsed and then conducted within each age group. The main locomotor behavior that correlations were attempted with was total distance traveled in the open field. No significant results were found between the total distance difference and D2 levels in either the NAc core or shell among any of the samples.

When age groups were combined, there were no linear regression equations that were significant at $p < .05$, but several trended toward significance. Equations could be written for D2 expression in both the core and the shell with time spent in the center of the OF. The equation relating core D2 mRNA expression and time in the center of the OF calculated as:

$$\text{Core} = .032 + (-3.551 \times 10^{-5} \text{ center time difference score})$$

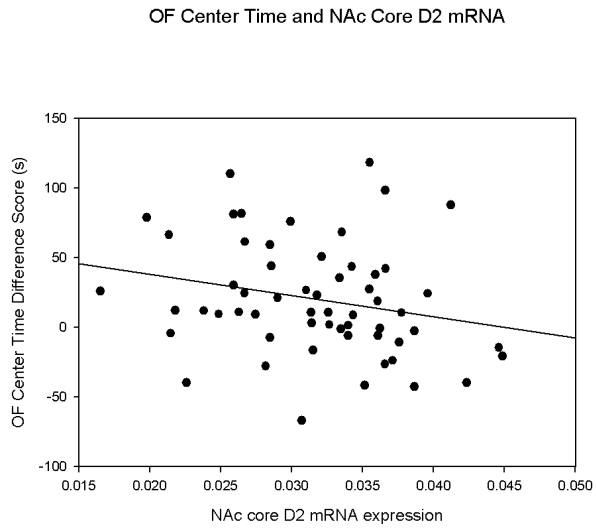


**Figure 10.** The relationship between NAc core D2 mRNA expression and OF center difference score trended toward significance when all subjects are compressed into one sample. The relationship is defined by the equation: core = .032 + (-3.551 x $10^{-5}$ center time difference score), R = .233, $p < .10$.

This model trends toward significance, F(1,57) = 3.202, R = .233, $p < .10$ (Fig 10). A similar relationship also exists between NAc shell D2 mRNA expression and OF center time, as expressed by the equation:

$$\text{Shell} = .030 + (-4.112 \times 10^{-5} \text{ center time difference score})$$

This relationship also trends toward significance, F(1,57) = 3.356, R = .238, $p < .10$ (Figure 11).

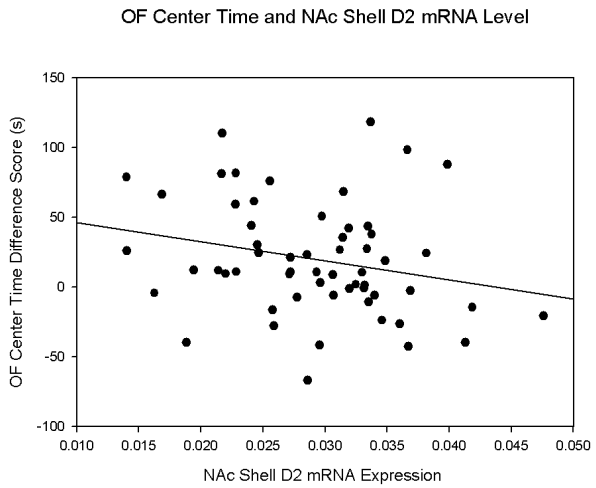


**Figure 11.** The relationship between NAc shell D2 mRNA expression and open field center time difference score when all subjects are compressed into one sample trended toward significance. The relationship for any individual is defined by the equation: shell = .030 + (-4.112 x $10^{-5}$ center time difference score), R = .238, $p < .10$.

Again, when age groups were combined, there was also a relationship that between NAc core D2 mRNA expression and time spent in the center of the EPM. This relationship is expressed by the equation:

$$\text{Core} = .034 + (-4.311 \times 10^{-5} \text{ center time})$$

This relationship also trended towards significance, F(1,57) = 2.920, R = .223, $p < .10$ (Figure 12). A similar relationship did not exist between the shell and EPM center time.

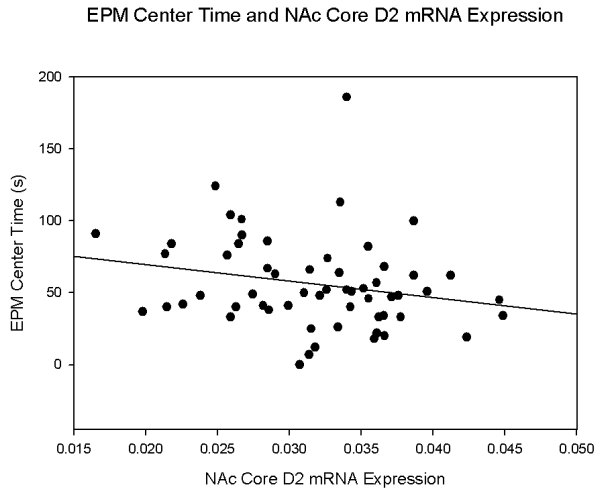


**Figure 12.** The relationship between NAc core D2 mRNA expression and time in the center of the EPM when all subjects were compressed into one sample trended toward significance. The relationship was expressed by the equation: core = .034 + (-4.311 x $10^{-5}$ center time), R = .233, $p < .10$.

The subject pool was also broken down by age groups for separate analyses. No significant relationships occurred among late adolescents, but two different types of significant relationships occurred among the early adolescents and the adults. Among early adolescents, EPM behavior was significantly related to core and shell D2 mRNA expression while in adults, open field behavior was better correlated to core and shell D2 expression.

Among early adolescents, only the core D2 expression was significantly correlated to EPM center time, $F(1,19) = 5.384$, $R = .480$, $p < .05$ (Figure 13). The relationship is defined by the equation:

Core = .040 + (.000 center time)

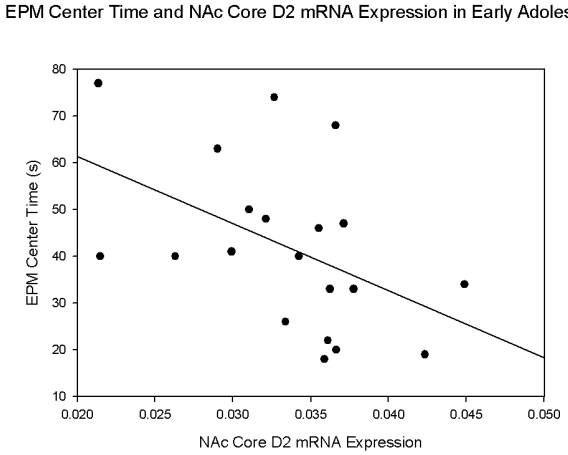


**Figure 13.** The relationship between NAc core D2 mRNA expression and center time in the EPM among early adolescents, R = .480, $p < .05$. This relationship is defined by the equation: core = .040 + (.000 center time).

Both the core and shell D2 expression were also significantly related to time spent in the open arms of the EPM. For the core, this was expressed by the equation:

$$\text{Core} = .037 + (-8.889 \times 10^{-5} \text{ open arm time})$$

These two variables were significantly correlated, $F(1,19) = 5.056$, R = .468, $p < .05$ (Figure 14). Shell D2 mRNA expression was also significantly related to open arm time, as expressed by the equation:

$$\text{Shell} = .035 + (-9.294 \times 10^{-5} \text{ open arm time})$$

and the correlation of the variables trended toward significance, F = 3.455, R = .401, $p < .10$ (Figure 15).

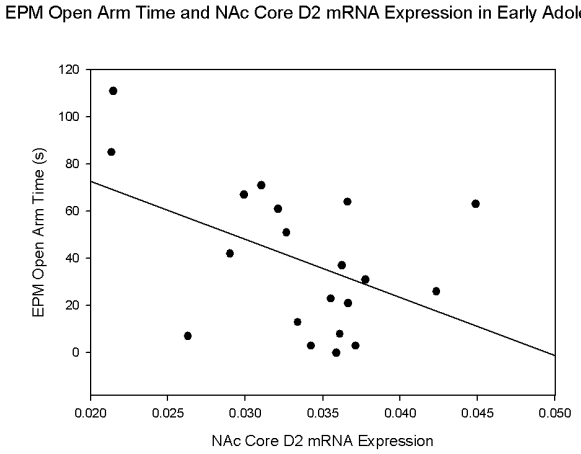


**Figure 14.** The relationship between NAc core D2 mRNA expression and time spent in the open arms on the EPM in early adolescents is significantly correlated, F = 5.056, R = .468, $p <$ .05 and expressed by the equation: core = .037 + (-8.889 x $10^{-5}$ open arm time).

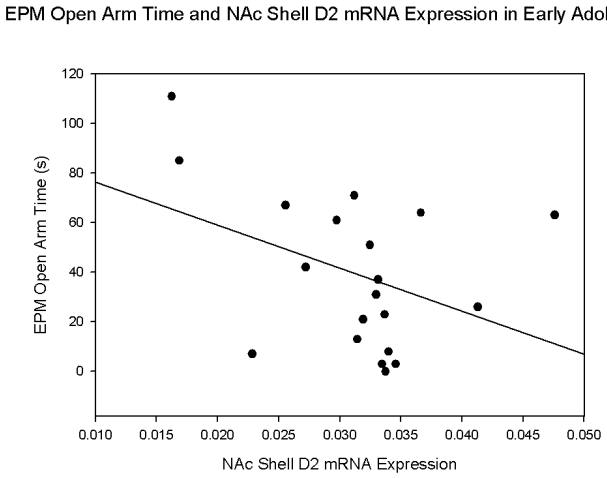


**Figure 15.** The relationship between NAc shell D2 mRNA expression and time spent in the open arms on the EPM in early adolescents trends toward significance, F= 3.455, R = .401, $p <$ .10 and is expressed by the equation: shell = .035 + (-9.294 x $10^{-5}$ open arm time).

Significant relationships among the adult group were between core and shell D2 expression with open field behavior, primarily distance traveled in the center and time

spent in the center, rather than EPM behaviors. Both the core and shell D2 mRNA levels significantly correlated with distance traveled in the center of the open field. The NAc core D2 mRNA expression had a significant relationship with the difference score of distance traveled in center of the open field, $F(1,19) = 6.373$, $R = 5.11$, $p < .05$ (Figure 16) and can be expressed by the following equation:

$$\text{Core} = .030 + (-1.521 \times 10^{-5} \text{ distance traveled in center difference score})$$

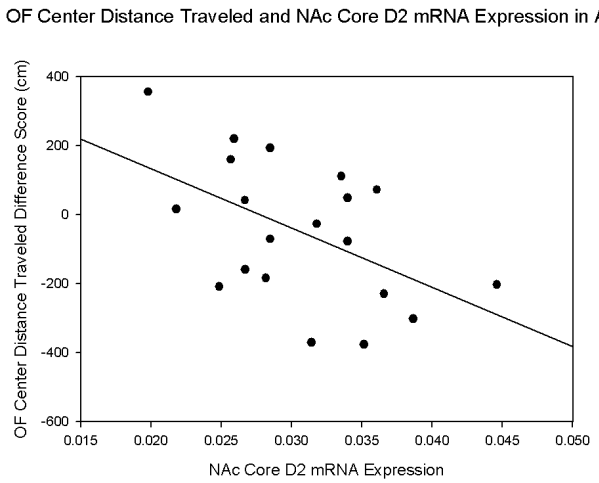


**Figure 16.** The relationship between NAc core D2 mRNA expression and distance traveled in the center of the open field in adults, $F = 6.373$, $R = .511$, $p < .05$. This relationship can be expressed by the equation core = $.030 + (-1.521 \times 10^{-5}$ center distance traveled difference score).

A similar relationship exists between distance traveled in the center of the OF and shell D2 expression, $F(1,19) = 7.395$, $R = .540$, $p < .05$ (Figure 17) and can be defined by the equation:

$$\text{Shell} = .027 + (-1.796 \times 10^{-5} \text{ distance traveled in center difference score})$$

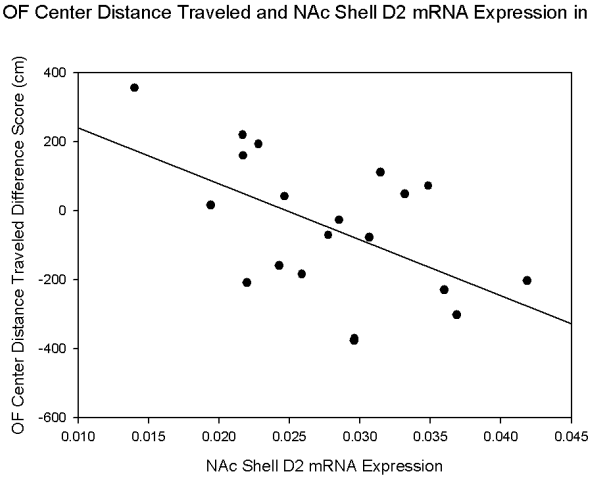


**Figure 17.** The relationship between NAc shell D2 mRNA expression and distance traveled in the center of the OF in adults, F = 7.395, R = .540, $p < .05$ and is defined by the equation: shell = .027 + (-1.796 x $10^{-5}$ distance travled in center difference score).

The D2 mRNA levels of both the core and shell are also both significantly correlated with time spent in the center of the OF. D2 expression in the core is significantly related to center time in the OF, F(1,19) = 7.201, R = .535, $p < .05$ (Figure 18) by the equation:

$$\text{Core} = .032 + (-7.758 \times 10^{-5} \text{ center time difference score})$$

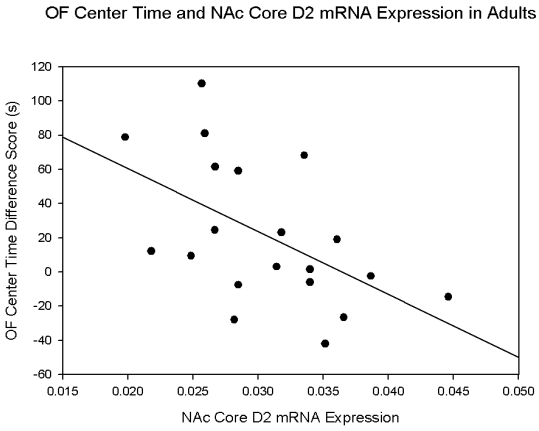


**Figure 18,** The relationship between NAc core D2 expression and time spent in the center of the OF in adults, F = 7.201, R = .535, $p < .05$ as expressed by: core = .032 + (-7.758 x $10^{-5}$ center time difference score).

A similarly significant relationship exists between D2 expression in the shell and center time in the OF, $F(1,19) = 8.832$, $R = .574$, $p < .01$ (Figure 19) and can be defined by the equation:

$$\text{Shell} = .030 + (-9.316 \times 10^{-5} \text{ center time difference score})$$

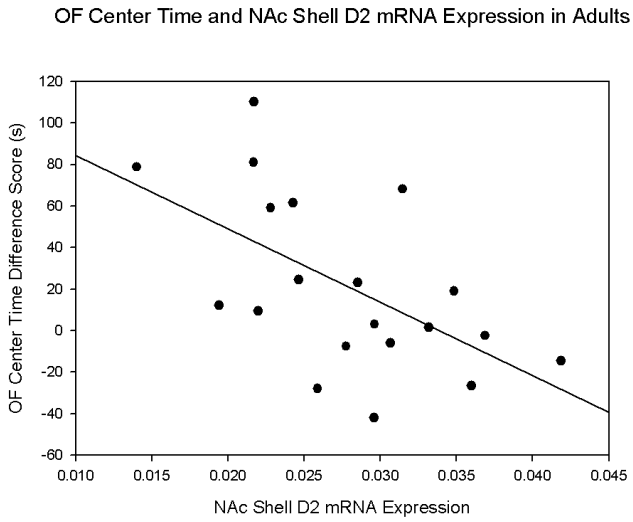


**Figure 19.** The relationship between NAc shell D2 mRNA expression and time spent in center of the open field in adults, $F = 8.832$, $R = .574$, $p < .01$ and can be defined by the equation shell = $.030 + (-9.316 \times 10^{-5}$ center time difference score).

## Summary

When the animals were tested in the EPM, the only significant finding was that adults spent significantly more time in the center than either early or late adolescents. There were no significant differences between age groups on the measures of open arm time, open arm entries, or protected head dips. When locomotor behavior was compared between a single nicotine injection and a baseline day, it was found that early adolescents were activated by the nicotine injection, while late adolescents and adults both exhibited

decreases in locomotor behavior. There were significant differences between early and late adolescents and early adolescents and adults on the measure of total distance traveled, and a significant difference between early adolescents and adults on the measure of distance traveled in the center. EPM behavior did not consistently correlate with OF behavior, with the exception of center time in the EPM and distance traveled in the center of the OF among early adolescents. HR/LR modeling was not consistently predictive of either locomotor or D2 relationships. The only significant finding was that LRs traveled a significantly greater distance than HRs among early adolescents. Finally, shifting relationships between locomotor behavior and D2 levels were found across the age groups. The EPM was more highly correlated with D2 levels in the core and shell among early adolescents and OF measures were more highly correlated with D2 levels in the core and shell among adults.

# DISCUSSION

Significant changes in behavioral variables and in NAc D2 mRNA expression were seen after a single injection of nicotine. Of particular interest, age was a mediating factor in results, though effects were seen at different ages for behavioral and neurochemical variables.

## EPM

The EPM was used as an anxiety pre-screening and, initially, the only measure that showed differences between age groups was center time with adults spending more time in the center of the EPM than either early or late adolescents. How representative this is of normal animals is difficult to ascertain since EPM is often used to measure anxiety after some type of modifying treatment and is commonly used to screen anxiolytic drugs (Ramos, 2008). Some reports have found that factor analyses show that entries into closed arms and open arms are best reflective of exploration (Boguszewski & Zagrodzka, 2002), though other work has shown significant differences between adolescents and adults on percentage of time spent in the center (Doremus, Varlinskaya, & Spear, 2006). It is also possible that use of the Long-Evans, which is relatively infrequently used in anxiety and drug testing, may account for this performance as not all strains perform the same in the EPM (Hogg, 1996). It is also difficult to determine

exactly what time in the center is measuring. It could be a measure of increased exploration since it is away from the closed arms the rat normally prefers, but it could still be a protected measure since the rat is not venturing out onto open arms. The EPM was not immediately predictive as an anxiety measure and did not particularly correlate with the OF, but it did play a large predictive role when paired together with D2 mRNA expression. This makes the EPM a potentially useful test as it can be correlated with neurochemistry.

OF

Two variables showed significant differences between age groups on the OF, total distance traveled and distance traveled in the center. These variables were calculated by subtracting Day 2 saline day values from Day 3 nicotine day values to create a difference score. On the total distance traveled variable, there were significant differences between the early and late adolescents and the early adolescents and adults. Of particular interest was the observation that early adolescents showed an overall increase in activity between Day 3 and Day 2 while late adolescents and adults showed decreases in distance traveled between testing and baseline days. A similar relationship held for distance traveled in the center. These findings suggest that both early and late adolescents are particularly susceptible to the locomotor effects of a single dose of nicotine, with early adolescents possessing a unique behavior pattern. Surprisingly, even though numerous papers show that adolescents have a distinctive vulnerability to drugs of abuse (Adriani, et al, 2003; Smith, 2003; Spear, 2000), few papers break adolescence into more than one group. Given the results in this project, including adolescence as a single age group could cloud

effects depending on the age examined. These results correspond with some findings already in the literature. Other work has found that adolescents do not show locomotor depressant effects to acute nicotine the way that adults do (Elliott, et al, 2004). Another study found activity depressant effects in adults and late adolescents after nicotine injection, but no activity effects in early adolescents (Belluzzi, et al, 2004). It is also possible that since only horizontal locomotor behaviors were measured, that there are competing locomotor behaviors (i.e., rearing, grooming, etc.) that are differing between nicotine and saline days that were not observed.

One part of the results that may be illuminating is the correlation between center time in the EPM and distance traveled in the center of the OF among early adolescents. Since these two behaviors are correlated, it may be that center time in the EPM is more of an exploration measure than a measure of anxiety-like behavior.

HR/LR Modeling

It is questionable how useful HR/LR modeling was in this study. Only total distance traveled in the OF was analyzed because this tends to be the behavior analyzed in other research. There were significant differences between HRs and LRs on this variable across the whole sample. However, it is likely that this effect is being dominated entirely by the strong effects in the early adolescents, which were the only age group that showed a significant effect between HRs and LRs. It is of interest that even though results were not significant in late adolescents and adults, all groups conformed to other work that shows that HRs show decreases in activity while LRs show less of a decrease or increases in activity (Rosecrans, 1995). Besheer and Bevins (2001) theorize that HRs

show greater sensitivity to nicotine locomotor suppressant effects because the HRs are starting at a higher activity level. It is likely this did not occur to the same extent in early adolescents in this cohort because they are not as susceptible to the locomotor suppressing effects of nicotine.

The HR/LR division was added to this cohort as a post hoc analysis. If the study were redesigned to take this model into account, larger cohorts could have been used and a top/bottom 33% methodology could have been utilized which may have better highlighted differences. Though it doesn't seem to appear in the literature, it might also be worth habituating animals in the OF in order to minimize the size of the changes between behavior after saline injection and after nicotine injection. Repeated nicotine exposures may also work better for this paradigm than a single exposure.

HR/LR modeling was utilized because it is a method that some studies use to address the issue of individual variance without going so far as to address each animal as a unique individual. Previous studies have often found that this method can predict future CPP and self-administration behavior, but the paradigm is not as clear when nicotine is used (Allen, et al, 2007; Deminière, et al, 1989; Rosecrans, 1995). HR/LR modeling was of particular interest because of the promising research relating HR/LR status to DA receptor and extracellular neurotransmitter levels (Hooks, et al, 1991b; Hooks, et al, 1994; Sabeti, et al, 2003).

*In Situ* Hybridization

D2 mRNA expression was significantly different between the NAc core and shell, allowing for them to be analyzed as individual regions. D2 agonists and antagonists have

been seen to influence the increased locomotion caused by nicotine (O'Neill, et al, 1991) and repeated dosing with nicotine has been shown to increase D2 mRNA in the NAc (Bahk, Li, Park, & Kim, 2002). Baseline locomotor behavior has also been shown to be predictive of D2 mRNA and extracellular dopamine levels in the NAc (Hooks, et al, 1991b; Hooks, et al, 1994). However, the correlation between D2 mRNA and locomotion has never been examined after a single injection of nicotine. It was thought that D2 mRNA would not change within 24 hours after nicotine injection and this would give the best measure of innate D2 receptor levels. One study showed increases of 75.4% in D2 mRNA after 10 minutes of exposure to smoking vapor for four weeks and of 39.2% after oral nicotine dosing (3 mg/kg/day) for four weeks (Bahk, et al, 2002). Clearly, D2 mRNA is altered in the NAc by nicotine administration, but there is no published research to suggest that it can be changed within a 24-hour period. Therefore, it is still assumed that this current study is an appropriate measure of innate D2 levels. In future work, it would be appropriate to add a control to firmly answer this question as it is not within the scope of this project to confirm or deny this assumption.

The sample was analyzed both as a whole and then broken down into separate ages, which allowed for predictive simple regression equations to be created relating D2 mRNA expression to various behavioral variables. These regression equations define the best-fit line for a given graph and relate the independent and dependent variable. Presumably, it is possibly to use this equation and substitute in the dependent variable (i.e., EPM center time or OF distance traveled in the center difference score) and predict the independent variable (the D2 mRNA level in the core or shell). However, there is so

much scatter off of the line of the plots (see Results section) that it is debatable how well this would really work.

Age groups were not compared to each other due to differences that exist in D2 mRNA during the ages being assessed (Creese, et al, 1992). It is particularly interesting that for early adolescents and adults equations could be calculated linking behavioral variables and D2 mRNA expression in the core and shell. In early adolescents, the EPM is a better predictive measure, correlating core and shell D2 expression with center time and open arm time. In adults, the OF is a better predictive measure, correlating core and shell D2 expression with center time and distance traveled in the center. These findings suggest the role of NAc D2 receptors in mediating locomotor behaviors may shift across the life span. Even though the predictive measures shift with age, it may be that these paradigms are still measuring similar attributes since the predictive variables mostly focus on center exploration.

Applications/Future Directions

It is possible that if these relationships hold up through further testing, this could be a useful animal model for examining relationships between addictive behaviors and D2 expression. D2 mRNA is expressed in roughly the same areas in humans and rats (Mengod, et al, 1992). D2 mRNA is indicative of actual receptor levels in the caudate-putamen and nucleus accumbens (Angulo, Coirini, Ledoux, & Schumacher, 1991). Human studies have shown that nicotine dependent subjects have significantly less striatal D2 receptor availability than those who have never smoked (Fehr, et al, 2008). Novelty-seeking, thought to be a trait of addictive behavior, has also been found to

negatively correlate with D2-like receptor availability (Zald, 2008). Studies have shown that users of numerous drugs of abuse, including nicotine, have decreased D2 receptor availability and decreased extracellular DA in the nucleus accumbens (Volkow, Fowler, Wang, Swanson, & Telang, 2007; Volkow, Fowler, Wang, Baler, & Telang, 2009). Volkow has suggested that higher D2 levels may be protective against drug self-administration (Volkow, et al, 2009). This study did not show negative correlations between locomotor behavior as a function of nicotine affect and D2 mRNA, but it is possible that further research using a similar model with repeated dosing may elucidate this relationship. Further exploration with a similar study may yield more information on novelty and the relationship to D2 and allow for better translational models.

As this is an exploratory study, it needs to be repeated in order to test the reliability of the findings, particularly the shifting predictive ability of the EPM and OF for the different age groups. It would also be useful to study D1 receptors as D2 and D1 are co-localized and D1 receptors also seem to play a role in nicotine-induced locomotion. Performing this experiment using repeated exposure rather than just a single dose may be of interest, though this dosing would significantly alter D2 mRNA from its initial point. It could also be interesting to add another adult time point as DA receptor levels keep changing up to about 120 days and this could further effect findings. Adding more animals may give further insight into why one test better predicts D2 expression for a particular age. Also, addition of a control group that did not receive nicotine injections would firmly ascertain whether DA mRNA could be affected in such a short time or is actually representative of baseline DA levels.

## Conclusion

This study showed differences between age groups on locomotor measures in the EPM and OF, continuing to show differences in locomotion and exploration between early and late adolescents and early adolescents and adults. It was also determined that behavior in the EPM was more highly correlated with NAc D2 mRNA levels among early adolescents and that behavior in the OF was more highly correlated with NAc D2 mRNA levels among adults. These results suggest that the role of NAc D2 receptors in mediating locomotor behavior may shift across the lifespan.

# REFERENCES

# REFERENCES


Adriani, W, Spijker, S, Deroche-Gamonet, V, Laviola, G, Le Moal, M, Smit, AB, et al. (2003). Evidence for enhanced neurobehavioral vulnerability to nicotine during periadolescence in rats. *The Journal of Neuroscience, 23*(11), 4712-4716.

Allen, RM, Everett, CV, Nelson, AM, Gulley, JM, & Zahniser, NR. (2007). Low and high locomotor responsiveness to cocaine predicts intravenous cocaine conditioned place preference in male Sprague-Dawley rats. *Pharmacology, Biochemistry and Behavior, 86,* 37-44.

Andersen, SL, Thompson, AT, Rutstein, M, Hotstetter, JC, & Teicher, MH. (2000). Dopamine receptor pruning in prefrontal cortex during the periadolescent period in rats. *Synapse, 37,* 167-169.

Angulo, JA, Coirini, H, Ledoux, M, & Schumacher, M. (1991). Regulation by dopaminergic neurotransmission of dopamine D2 mRNA and receptor levels in the striatum and nucleus accumbens of the rat. *Molecular Brain Research, 11,* 161-166.

Anthony, JC, Warner, LA, & Kessler, RC. (1994). Comparative epidemiology of dependence on tobacco, alcohol, controlled substances, and inhalants: basic findings from the national comorbidity survey. *Experimental Clinical Psychopharmacology, 2,* 244-268.

Badanich, KA, & Kirstein, CL. (2004). Nicotine administration significantly alters accumbal dopamine in the adult but not in the adolescent rat. *Annals of the New York Academy of Science, 1021,* 410-417.

Bahk, JY, Li, S, Park, MS, & Kim, MO. (2002). Dopamine D1 and D2 receptor mRNA up-regulation in the caudate-putamen and nucleus accumbens of rat brains by smoking. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 26,* 1095-1104.

Balfour, DJK, Benwell, MEM, Birrell, CE, Kelly, RJ, & Al-Aloul, M. (1998). Sensitization of the mesoaccumbens dopamine response to nicotine. *Pharmacology, Biochemistry and Behavior, 59*(4), 1021-1030.

Belluzzi, JD, Lee, AG, Oliff, HS, & Leslie, FM. (2004). Age-dependent effects of nicotine on locomotor activity and conditioned place preference in rats. *Psychopharmacology, 174*, 389-395.

Benwell, MEM, & Balfour, DJK. (1992). The effects of acute and repeated nicotine treatment on nucleus accumbens dopamine and locomotor activity. *British Journal of Pharmacology, 105*, 849-856.

Benwell, MEM, Balfour, DJK, & Khadra, LF. (1994). Studies on the influence of nicotine infusions on mesolimbic dopamine and locomotor responses to nicotine. *Clinical Pharmacology, 72*, 233-239.

Bevins, RA, & Besheer, J. (2001). Individual differences in rat locomotor activity are diminished by nicotine through stimulation of central nicotinic acetylcholine receptors. *Physiology & Behavior, 72*, 237-244.

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

Boye, SM, Grant, RJ, & Clarke, PBS. (2001). Disruption of dopaminergic neurotransmission in nucleus accumbens core inhibits the locomotor stimulant effects of nicotine and d-amphetamine in rats. *Neuropharmacology, 40*, 792-805.

Breslau, N, & Peterson, EL. (1996). Smoking cessation in young adults: Age at initiation of cigarette smoking and other suspected influences. *American Journal of Public Health, 86*(2), 214-220.

Briegleb, SK, Gulley, JM, Hoover, BR, & Zahniser, NR. (2004). Individual differences in cocaine- and amphetamine-induced activation of male Sprague-Dawley rats: Contribution of the dopamine transporter. *Neuropsychopharmacology, 29*, 2168-2179.

Brody, AL, Mandelkern, MA, Olmstead, RE, Scheibal, D, Hahn, E, Shiraga, S, et al. (2006). Gene variants of brain dopamine pathways and smoking-induced dopamine release in the ventral caudate/nucleus accumbens. *Archives of General Psychiatry,63*, 808-816.

Canales, JJ, & Iversen, SD. (2000). Psychomotor-activating effects mediated by dopamine D2 and D3 receptors in the nucleus accumbens. *Pharmacology, Biochemistry and Behavior, 67*, 161-168.

Cao, J, Belluzzi, JD, Loughlin, SE, Keyler, DE, Pentel, PR, & Leslie, FM. (2007). Acetaldehyde, a major constituent of tobacco smoke, enhances behavioral, endocrine, and neuronal responses to nicotine in adolescent and adult rats. *Neuropsychopharmacology, 32,* 2025-2035.

Carey, RJ, DePalma, G, & Damianopoulos, E. (2003). Response to novelty as a predictor of cocaine sensitization and conditioning in rats: a correlational analysis. *Psychopharmacology, 168,* 245-252.

Carlezon, WA, & Thomas, MJ. (2009). Biological substrates of reward and aversion: A nucleus accumbens activity hypothesis. *Neuropharmacology, 56,* 122-132.

Chen, J, & Millar, WJ. (1998). Age of smoking initiation: Implications for quitting. *Health Reports, 9*(4), 39-46.

Clarke, PBS, Fu, DS, Jakubovic, A, & Fibiger, HC. (1988). Evidence that mesolimbic dopaminergic activation underlies the locomotor stimulant action of nicotine in rats. *The Journal of Pharmacology and Experimental Therapeutics, 246*(2), 701-708.

Collins, SL, Wade, D, Ledon, J, & Izenwasser, S. (2004). Neurochemical alterations produced by daily nicotine exposure in periadolescent vs. adult male rats. *European Journal of Pharmacology, 502,* 75-85.

Creese, I, Sibley, DR, & Xu, SX. (1992). Expression of rat striatal D1 and D2 dopamine receptor mRNAs: Ontogenetic and pharmacological studies. *Neurochemistry International, 20 Suppl,* 45S-48S.

Cruz, F, DeLucia, R, & Planeta, CS. (2005). Differential behavioral and neuroendocrine effects of repeated nicotine in adolescent and adult rats. *Pharmacology, Biochemistry and Behavior, 80,* 411-417.

Dadmarz, M, & Vogel, WH. (2003). Individual self-administration of nicotine by rats. *Pharmacology, Biochemistry and Behavior, 76,* 425-432.

Davis, BA, Clinton, SM, Akil, H, & Becker, JB. (2008). The effects of novelty-seeking phenotypes and sex differences on acquisition of cocaine self-administration in selectively bred High-Responder and Low-Responder rats. *Pharmacology, Biochemistry and Behavior, 90,* 331-338.

Deminière, JM, Piazza, PV, Le Moal, M, & Simon, H. (1989). Experimental approach to individual vulnerability to psychostimulant addiction. *Neuroscience & Biobehavioral Reviews, 13,* 141-147.

DiChiara, G. (2000). Role of dopamine in the behavioural actions of nicotine related to addiction. *European Journal of Pharmacology, 393,* 295-314.

Doremus, TL, Varlinskaya, EI, & Spear, LP. (2006). Factor analysis of elevated plus-maze behavior in adolescent and adult rats. *Pharmacology, Biochemistry and Behavior, 83*, 570-577.

Elliott, BM, Faraday, MM, Phillips, JM, & Grunberg, NE. (2004). Effects of nicotine on elevated plus maze and locomotor activity in male and female adolescent and adult rats. *Pharmacology, Biochemistry and Behavior, 77,* 21-28.

Erb, SM, & Parker, LA. (1994). Individual differences in novelty-induced activity do not predict strength of amphetamine-induced place conditioning. *Pharmacology, Biochemistry and Behavior, 48*(3), 581-586.

Faraday, MM, Elliott, BM, & Grunberg, NE. (2001). Adult vs. adolescent rats differ in biobehavioral responses to chronic nicotine administration. *Pharmacology, Biochemistry and Behavior, 70*, 475-489.

Faraday, MM, Elliott, BM, Phillips, JM, & Grunberg, NE. (2003). Adolescent and adult male rats differ in sensitivity to nicotine's activity effects. *Pharmacology, Biochemistry and Behavior, 74*, 917-931.

Fehr, C, Yakushev, I, Hohmann, N, Buchholz, H, Landvogt, C, Deckers, H, et al. (2008). Association of low striatal dopamine D2 receptor availability with nicotine dependence similar to that seen with other drugs of abuse. *The American Journal of Psychiatry, 165*, 507-514.

Gong, W, Neill, DB, & Justice, JB. (1996). Locomotor response to novelty does not predict cocaine place preference conditioning in rats. *Pharmacology, Biochemistry and Behavior, 53,* 191-196.

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

Hooks, MS, Jones, GH, Smith, AD, Neill, DB, & Justice, JB. (1991a). Individual differences in locomotor activity and sensitization. *Pharmacology, Biochemistry and Behavior, 38,* 467-470.

Hooks, MS, Jones, GH, Smith, AD, Neill, DB, & Justice, JB. (1991b). Response to novelty predicts the locomotor and nucleus accumbens dopamine response to cocaine. *Synapse, 9,* 121-128.

Hooks, MS, Juncos, JL, Justice, JB, Meiergerd, SM, Povlock, SL, Schenk, JO, et al. (1994). Individual locomotor response to novelty predicts selective alterations in D1 and D2 receptors and mRNAs. *The Journal of Neuroscience, 14*(10), 6144-6152.

Ikemoto, S, Glazier, BS, Murphy, JM, & McBride, WJ. (1997). Role of dopamine D1 and D2 receptors in the nucleus accumbens in mediating reward. *The Journal of Neuroscience, 17*(21),8580-8587.

Imperato, A, Mulas, A, & DiChiara, G. (1986). Nicotine preferentially stimulates dopamine release in the limbic system of freely moving rats. *European Journal of Pharmacology, 132*, 337-338.

Johnson, JH, Zhao, C, James, JR, & Rosecrans, JA. (2000). Individual variability of dopamine release from nucleus accumbens induced by nicotine. *Brain Research Bulletin, 51*(3), 249-253.

Kabbaj, M. (2006). Individual differences in vulnerability to drug abuse: the high responders/low responders model. *CNS & Neurological Disorders-Drug Targets, 5*, 513-520.

Kandel, DB, & Chen, K. (2000). Extent of smoking and nicotine dependence in the United States: 1991-1993. *Nicotine & Tobacco Research, 2,* 263-274.

Koob, GF, & Le Moal, M. (2008). Addiction and the brain antireward system. *The Annual Review of Psychology, 59,* 29-53.

Laviolette, SR, Lauzon, NM, Bishop, SF, Sun, N, & Tan, H. (2008). Dopamine signaling through D1-like versus D2-like receptors in the nucleus accumbens core versus shell differentially modulates nicotine reward sensitivity. *Journal of Neuroscience, 28*(32),8025-8033.

Mandt, BH, Schenk, S, Zahniser, NR, & Allen, RM. (2008). Individual differences in cocaine-induced locomotor activity in male Sprague-Dawley rats and their acquisition of and motivation to self-administer cocaine. *Psychopharmacology, 201*(2), 195-202.

Mengod, G, Villaró, MT, Landwehrmeyer, GB, Martinez-Mir, MI, Niznik, HB, Sunahara, RK, et al. (1992). Visualization of dopamine D1, D2, and D3 receptor mRNAs in human and rat brain. *Neurochemical International, 20Suppl*, 33S-43S.

Nisell, M, Marcus, M, Nomikos, GG, & Svensson, TH. (1997). Differential effects of acute and chronic nicotine on dopamine output in the core and shell of the rat nucleus accumbens. *Journal of Neural Transmission, 104,* 1-10.

O'Neill, MF, Dourish, CT, & Iversen, SD. (1991). Evidence for an involvement of D1 and D2 dopamine receptors in mediating nicotine-induced hyperactivity in rats. *Psychopharmacology, 104,* 343-350.

Paxinos, G, & Watson, C. (1998). *The rat brain: In stereotaxic coordinates* (4th ed.). San Diego: Academic.

Perkins, KA. (1995). Individual variability in responses to nicotine. *Behavior Genetics, 25*(2), 119-132.

Piazza, PV, Deminière, JM, Le Moal, M, & Simon, H. (1989). Factors that predict individual vulnerability to amphetamine self-administration. *Science, 245,* 1511-11513.

Pontieri, FE, Tanda, G, Orzi, F, & DiChiara, G. (1996). Effects of nicotine on the nucleus accumbens and similarities to those of addictive drugs. *Nature, 382,* 255-257.

Ramos, A. (2008). Animal models of anxiety: Do I need multiple tests? *Trends in Pharmacological Sciences, 29,* 493-498.

Rose, JE. (2006). Nicotine and nonnicotine factors in cigarette addiction. *Psychopharmacology, 184,* 274-285.

Rosecrans, JA. (1971). Effects of nicotine on behavioral arousal and brain 5-hydroxytryptamine function in female rats selected for differences in activity. *European Journal of Pharmacology, 14,* 29-37.

Rosecrans, JA. (1995). The psychopharmacological basis of nicotine's differential effects on behavior: Individual subject variability in the rat. *Behavior Genetics, 25*(2), 187-196.

Sabeti, J, Gerhardt, GA, & Zahniser, NR. (2003). Individual differences in cocaine-induced locomotor sensitization in low and high cocaine locomotor-responding rats are associated with differential inhibition of dopamine clearance in nucleus accumbens. *Journal of Pharmacology and Experimental Therapeutics, 305,* 180-190.

Schochet, TL, Kelley, AE, & Landry, CF. (2004). Differential behavioral effects of nicotine exposure in adolescent and adult rats. *Psychopharmacology, 175,* 265-273.

Sellings, LHL, Baharnouri, G, McQuade, LE, & Clarke, PBS. (2008). Rewarding and aversive effects of nicotine are segregated within the nucleus accumbens. *European Journal of Neuroscience, 28,* 342-352.

Shearman, E, Fallon, S, Sershen, H, & Lajtha, A. (2008). Nicotine-induced monoamine neurotransmitter changes in the brain of young rats. *Brain Research Bulletin, 76,* 626-639.

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

Spear, L. (2000). Modeling adolescent development and alcohol use in animals. *Alcohol Research & Health, 24*(2), 115-123.

Trauth, JA, Seidler, FJ, & Slotkin, TA. (2000). Persistent and delayed behavioral changes after nicotine treatment in adolescent rats. *Brain Research, 880,* 167-172.

Trauth, JA, Seidler, FJ, Ali, SF, & Slotkin, TA. (2001). Adolescent nicotine exposure produces immediate and long-term changes in CNS noradrenergic and dopaminergic function. *Brain Research, 892,* 269-280.

Teicher, MH, Andersen, SL, & Hostetter, JC. (1995). Evidence for dopamine receptor pruning between adolescence and adulthood in striatum but not nucleus accumbens. *Developmental Brain Research, 89,* 167-172.

Torres, OV, Tejeda, HA, Natividad, LA, & O'Dell LE. (2008). Enhanced vulnerability to the rewarding effects of nicotine during the adolescent period of development. *Pharmacology, Biochemistry and Behavior, 90,* 658-663.

Volkow, ND, Fowler, JS, Wang, G-J, Swanson, JM, & Telang, F. (2007). Dopamine in drug abuse and addiction. *Archives of Neurology, 64,* 1575-1579.

Volkow, ND, Fowler, JS, Wang, G-J, Baler, R, & Telang, F. (2009). Imaging dopamine's role in drug abuse and addiction. *Neuropharmacology, 56,* 3-8.

Wise, RA, & Bozarth, MA. (1987). A psychomotor stimulant theory of addiction. *Psychological Review, 94,* 469-492.

Young, W.S., III. (1992). In situ hybridization with oligodeoxyribonucleotide probes. In: Wilkinson, D.G. ed. *In Situ Hybridization: A Practical Approach.* New York: Oxford University Press, p.33-44.

Zald, DH, Cowan, RL, Riccardi, P, Baldwin, RM, Ansari, MS, Li, R, et al. (2008). Midbrain dopamine receptor availability is inversely associated with novelty-seeking traits in humans. *The Journal of Neuroscience, 28,* 14372-14378.

# CURRICULUM VITAE

Adriana M. Falco graduated from La Plata High School, La Plata, Maryland, in 1999. She received her Bachelor of Science from University of Maryland—College Park in 2003. Her research has focused on drug abuse animal models, particularly in adolescent nicotine use.