INDIVIDUAL DIFFERENCES IN LOCOMOTION, ANXIETY-LIKE BEHAVIOR, AND REWARD AFTER NICOTINE AND BACLOFEN ADMINISTRATION

by

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Individual Differences in Locomotion, Anxiety-like behavior, and Reward After Nicotine and Baclofen Administration

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DEDICATION

This is dedicated to the memory of my brother, Nicholas R. Falco, III.

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I would like to thank the many friends, relatives, and supporters who have made this project possible. My parents, Nicholas Falco, Patricia Pruss, and Frank Pruss have been supportive throughout my graduate career. Drs. Smith, McDonald, and Fryxell, as members of my committee, all gave invaluable research guidance. Finally, thanks go to Charles Blanchard and Gina Fernandez who gave technical assistance during the project.

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ABSTRACT

INDIVIDUAL DIFFFERENCES IN LOCOMOTION, ANXIETY-LIKE BEHAVIOR, AND REWARD AFTER NICOTINE AND BACLOFEN ADMINISTRATION

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George Mason University, 2012

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Tobacco use is a significant health problem that began in adolescence for many adult smokers. Anxiety may also be a risk factor in who develops nicotine dependence disorders. This study uses adolescent male Sprague-Dawley rats (n = 160) and splits them into a high anxiety (HA) and low anxiety (LA) group based on the results of pretest day of a conditioned place preference (CPP) protocol with a biased chamber. These rats are further divided into drug groups that receive either saline or 0.6 mg/kg baclofen (i.p.) 30 minutes before testing and then either saline or 0.5 mg/kg nicotine (s.c.) immediately before testing.

Open field testing showed a significant difference between HA and LA rats in locomotor activity, as well as significant differences between drug groups when compared to saline. Notably, baclofen administration significantly decreased locomotor behavior from saline levels in HA animals, but did not do so in LA animals. In both HA

and LA groups, baclofen and nicotine co-administration significantly decreased locomotor behavior from locomotor activity levels in animals administered nicotine alone. Additionally, the open field was used to examine potential differences in anxiety-like behavior. Baclofen administration failed to produce differences in anxiety-like behavior between HA and LA groups, but nicotine administration and baclofen + nicotine co-administration had slightly more of an effect on anxiety-like behavior in LA than HA animals. Single-trial nicotine CPP testing found that HA rats formed significant CPP to nicotine and baclofen + nicotine, but LA rats did not. This study shows that innate anxiety-like behavior plays a significant factor in formation of locomotor responses to baclofen as well as later anxiety-like responses to nicotine and baclofen administration in adolescent rats. This study also serves to highlight the role that innate anxiety-like behavior plays in nicotine reward in adolescents.

INTRODUCTION

Tobacco use represents a serious health epidemic, constituting the leading preventable cause of premature death (US Health and Human Services, 2010). Of particular interest are the prevention of and/or intervention in nicotine dependence disorders prior to costly outcomes. Adolescence forms a unique period of vulnerability to nicotine. The majority of smokers begin smoking prior to age 17 and demonstrate a decreased ability to quit smoking as compared to smokers who begin smoking later in life (Breslau & Peterson, 1996; Chen & Millar, 1998). Adolescent smokers also report higher levels of tolerance and dependence than adult counterparts (Kandel & Chen, 2000). Research with rodent models also supports the risk of adolescents to the development of nicotine dependence and addiction. The rewarding effects of nicotine are heightened in adolescent rats, marking a critical period for the development of nicotine dependence (Adriani, et al., 2003; Belluzzi, Lee, Oliff, & Leslie, 2004; Brielmaier, McDonald, Smith, 2007; Torres, Tejada, Natividad, & O'Dell, 2008).

The coexistence of anxiety disorders and substance use disorders is present in numerous populations, including adolescents. However, the direction of causation of

anxiety disorders and substance use disorders has yet to be clearly ascertained. Human research has noted that significantly higher percentages of individuals with anxiety disorders will develop substance dependence disorders than those in the general population (Liang, Chikritzhs, & Lenton, 2011). Adolescents who report social fears and social anxiety have a significantly higher risk of using cigarettes and developing nicotine dependence (Henry, Jamner, & Whalen, 2012; McKenzie, Olsson, Jorm, Romaniuk, & Patton, 2010; Sonntag, Wittchen, Höfler, Kessler, & Stein, 2000). Rates of social anxiety or generalized anxiety disorder are also correlated with an earlier age of first tobacco use (Mamorstein, White, Loeber, & Stouthamer-Loeber, 2010). Research with rodent models has also investigated the impact of anxiety-like behavior on reward, mainly in adulthood, with unclear results. When age is condensed into a homogenous group, one study found that low anxiety-like behavior predicted higher levels of drug seeking in cocaine self-administration (Schramm-Sapyta, et al., 2011). However, other work has found that high anxiety-like behavior is associated with greater intake of cocaine in self-administration and increases in place conditioning stimulated by cocaine (Dilleen, et al., 2012; Pelloux, Costentin, & Duterte-Boucher, 2009).

Anxiety and substance use disorders may be comorbid, but the underlying pathophysiology that links them has yet to be determined. Dysfunction of the γ-aminobutyric acid (GABA) system has been implicated in both anxiety (Millan, 2003) and substance use and abuse (Heilig, Goldman, Berrettini, & O'Brien, 2011; Shorter & Kosten, 2011). The metabotropic $GABA_B$ receptor has been of particular research interest in both anxiety disorders (Mombereau, et al., 2004; Ong & Kerr, 2005; Partyka,

et al., 2007) and drug addiction (Bowery, 2006; Cousins, Roberts, & de Wit, 2002; Tyacke, Lingford-Hughes, Reed, & Nutt, 2010). To date, the specific roles that $GABA_B$ receptors play in these disorders has not been elucidated. One drug under investigation for both anxiety and substance abuse disorders is baclofen, a $GABA_B$ agonist currently approved by the U.S. Food and Drug Administration (FDA) to treat muscle spasticity (US Food and Drug Administration, 2011). There is scant research addressing baclofen's involvement in anxiety and anxiety-like behavior. Research has shown that baclofen administration has anxiolytic effects in the elevated plus maze (EPM) in male mice (Amikishieva & Semendyaeva, 2007), but fails to modify nicotine-induced anxiety-like behavior in mice (Varani & Balerio, 2012).

The effects of baclofen on drug addiction have been far better addressed, both in clinical and preclinical populations. The use of baclofen in clinical populations has highlighted a potential role for its use in the treatment of drug addiction and substance use disorders. Baclofen has been found to alter the sensory aspects of smoking, decreasing the enjoyment of cigarettes (Cousins, Stamat, & de Wit, 2001) as well as reducing the number of cigarettes smoked (Franklin, et al., 2009). Baclofen administration has also been found to decrease daily alcohol intake among alcoholics (Addolorato, et al., 2011), in addition to reducing craving and withdrawal symptoms (Addolorato & Leggio, 2010). There has also been some implication that baclofen may be useful in decreasing craving in some cocaine dependent subjects (Haney, Hart, & Foltin, 2006).

Work with preclinical samples is also showing promise for the use of baclofen as a treatment for drug dependence and addiction. The acute administration of baclofen in Sardinian alcohol-preferring rats was found to suppress extinction phase responding for alcohol in a two bottle choice paradigm (Colombo, et al., 2003). Direct intracerebral injections of baclofen into the ventral tegmental area (VTA) of rats were found to reduce cocaine self-administration (Brebner, Childress, & Roberts, 2002). Baclofen administration has also been found to prevent reinstatement of heroin (Spano, Fattore, Fratta, & Fadda, 2007) and nicotine (Fattore, et al., 2009) self-administration as well as reducing rates of nicotine self-administration (Fattore, Cossu, Martellotta, & Fratta, 2002; Paterson, Forestl, & Markou, 2004). Baclofen pretreatment has also been shown to block nicotine conditioned place preference (CPP) effects (Le Foll, Wertheim, & Goldberg, 2008) and enhance extinction of morphine CPP (Heinrichs, Leite-Morris, Carey, & Kaplan, 2010). In addition, pretreatment with baclofen was found to attenuate sensitization and locomotor effects of cocaine (Frankowska, Nowak, & Filip, 2009), amphetamine (Bartoletti, Gubellini, Ricci, & Gaiardi, 2005), morphine (Bartoletti, Ricci, & Gaiardi, 2007), and nicotine (Lobina, et al., 2011; Palmatier & Bevins, 2002).

While baclofen is generally considered a safe substance with limited abuse potential (Evans & Bisaga, 2009), there have been cases of baclofen overuse and abuse (Dore, Lo, Juckes, Bezyan, & Latt, 2011; May, 1983; Nasti & Brakoulias, 2011; Perry, Wright, Shannon, & Woolf, 1998). Some of the previous citations show some evidence that anxious users may be at a higher risk to abuse baclofen due its anxiolytic effects, which have led to cases of overuse and abuse. Baclofen overdose is known to cause

numerous ill effects, including seizures, coma, and delirium (Chong & Wang, 2005; Wall, Wasiak, & Hicklin, 2006).

The present study examined the effects of acute doses of baclofen and nicotine on locomotion, anxiety-like behavior, and reward in adolescent male Sprague-Dawley rats that were split into high anxiety (HA) and low anxiety (LA) groups based on pretesting with a biased CPP apparatus. Locomotor and anxiety-like behavior were assessed in the open field (OF) while reward was measured via single-trial nicotine CPP using a "biased" methodology. It was hypothesized that administration of baclofen would cause differences in anxiety-like behavior, with HA animals showing larger changes in anxiety-like behavior due to higher initial anxiety levels. In addition, it was hypothesized that baclofen administration would increase rates of single-trial nicotine CPP due to possible anxiolytic reduction of baclofen seen in humans.

MATERIALS AND METHODS

Animals

Male adolescent Sprague-Dawley rats (n = 160) were obtained from Harlan (Indianapolis, IN, USA) and housed in groups of four or five on a 12 h light/12 h dark schedule (lights on at 0700). Food and water were available *ad libitum*, with animals being given additional food pulp (chow mixed with water) at arrival to supplement the diet. Subjects were acclimatized to the colony for seven days prior to testing. Behavioral testing began at postnatal day 28 (P28). All experiments were approved by the George Mason University animal care committee and in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (2011).

Drugs

(-)-Nicotine hydrogen tartrate and R (+) baclofen hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO). All drugs were administered at an injection volume of 1 mL/kg body weight. Baclofen and saline were administered intraperitoneally (i.p.), and nicotine and saline were administered subcutaneously (s.c.) between the shoulder blades for both open field and CPP experiments. Baclofen and

nicotine were dissolved in saline solution (0.9% NaCl). Dose levels of nicotine are expressed as free base equivalent, and the pH was adjusted to 7.1-7.4.

Pretesting

Animals were divided into HA and LA groups on the basis of pretesting. Pretesting utilized the CPP chamber to determine innate levels of anxiety-like behavior. The chamber is akin to the light-dark box, a well-known apparatus for testing anxiety-like behavior, in that it is composed of a black chamber and a white chamber. Similarly to the light-dark box, animals spend varying amounts of time in the white chamber; those that spend more time in the white chamber were considered to have low anxiety, those that spend less time in the white chamber were considered to have high anxiety.

The first day of behavioral testing for all animals consisted of pretesting in order to divide into high or low anxiety groups based on median split within each drug group. Animals were given access to both sides of the apparatus for 15 minutes and after testing, animals were divided into HA and LA animals and utilized in the next portions of testing.

Open Field (OF)

Apparatus

Locomotor and anxiety-like behavioral testing was performed in four OF chambers, created from white Plexiglas, measuring 42 x 42 x 30 cm, and located in a dimly lit (4-6 lx) testing room. A camera mounted above the apparatus recorded the 15-minute trials and data were acquired in 3 x 5-minute intervals using Videotrack software (Viewpoint, Montreal, QC, Canada). Between each set of animals, each chamber was cleaned with 70% EtOH to eliminate odor cues.

Procedure

Eighty animals from pretesting divided into the following groups: HA (n = 40) and LA (n = 40). Each group was further split into one of the following four drug treatment groups: saline+saline, saline + nicotine, baclofen + saline, and baclofen + nicotine (see Table 1). Therefore, each drug treatment group consisted of a HA (n =10) and a LA (n =10) component.

On test days, animals were housed in individual hanging wire cages and permitted to habituate to the testing room for 20 minutes. Thirty minutes prior to OF, rats were injected (i.p.) with either baclofen or saline, depending on the treatment group. Immediately before testing, another injection, of either nicotine or saline (s.c.) was given, again depending on the treatment group. After drug injections, animals were tested in the OF chamber for 15 minutes, with data being collected over 3 x 5 minute intervals.

Place Conditioning

Apparatus

Conditioned place preference (CPP) testing occurred in a two chambered apparatus (Med Associates, VT) in the same testing room as OF. Each chamber of the apparatus consisted of Plexiglas and had dimensions 21 x 42 x 30 cm. One chamber consisted of white walls with a mesh floor over a white paper lining, while the opposite chamber consisted of black walls with a stainless steel rod floor over a black paper lining. A black removable door separated the two chambers.

Table 1. List of drug group denotations for open field (OF).

Label	1st Injection	2nd Injection
saline + saline	Saline	saline
baclofen + saline	Baclofen	saline
saline + nicotine	Saline	nicotine
baclofen + nicotine	Baclofen	nicotine

Procedure

Eighty animals from pretesting were split into HA (n =40) and LA (n = 40) groups and were further designated to received (saline + saline)CPP, (baclofen + saline)CPP, (saline + nicotine)CPP, or (baclofen + nicotine)CPP during CPP testing (see Table 2). All animals underwent single-trial nicotine CPP testing in a "biased" place conditioning method modified from previous Smith lab protocols (Brielmaier, McDonald, & Smith, 2007; Brielmaier, McDonald, & Smith, 2008). In a "biased" procedure, animals were tested for their natural preference to a chamber and then conditioned with a drug in the non-preferred chamber. Testing consisted of three aspects: pretest, conditioning sessions, and posttest. Each day, animals were placed into individual wire hanging cages and permitted to habituate to the testing room for 20 minutes prior to testing. On the pretest day, each animal was placed in the apparatus and given free access to both chambers for 15 minutes. Natural, or unconditioned, preference for a chamber was determined by recording the amount of time spent in the white chamber. The definition of time spent in the white chamber was described as when the rat had all 4 paws completely in the white chamber. All rats were started in the white chamber, facing toward the removable door.

Animals underwent two conditioning sessions, one to administer drug (or saline in the case of controls), and one where all animals received saline. Animals were counterbalanced so that half received drug on the first conditioning session (and saline in the second), and half received drug in the second conditioning session (and saline in the first). During conditioning sessions, animals were weighed before being placed in the

Table 2. List of drug group denotations in conditioned place preference (CPP).

Label	Nonpreferred Chamber--1st Injection	Nonpreferred Chamber--2nd Injection	Preferred Chamber--1st Injection	Preferred Chamber—2nd Injection
(saline+saline)CPP	Saline	saline	saline	saline
(baclofen+saline)CPP	Baclofen	saline	saline	saline
(saline+nicotine)CPP	Saline	nicotine	saline	saline
(baclofen+nicotine)CPP	Baclofen	nicotine	saline	saline

hanging cages and habituated. On drug conditioning days, animals then received an injection of either saline or 0.6 mg/kg R (+) baclofen (i.p.) and waited a period of 30 minutes. Immediately prior to CPP, animals received an injection of either saline or 0.5 mg/kg nicotine (s.c.) and were placed in their initially non-preferred chamber, facing away from the door, for 15 minutes. On saline conditioning days, all animals received an injection of saline (i.p.) 30 minutes before CPP, and then an injection of saline (s.c.) immediately prior to CPP testing and were placed in their initially preferred chamber, facing away from the door, for 15 minutes.

On the posttest day, animals were again given free access to the testing apparatus to determine chamber preference during a 15-minute drug-free posttest. All animals were again started in the white chamber facing the removed door. Preference was determined by time spent in the white chamber. Between all trials, both chambers were cleaned with 70% EtOH and paper was changed after each animal to remove odors.

Statistics

Locomotor and anxiety-like behavioral variables were analyzed in quantified records of OF activity using simple regressions with dummy coding in order to take into account the categorical variables. For the CPP experiment, difference scores were calculated for each animal by subtracting time in seconds spent in the initially non-preferred chamber on the posttest day from time in seconds spent in the initially non-preferred chamber on the pretest day. Again, simple regressions with dummy coding were conducted to analyze data and take into account the categorical variables. Regressions were used over ANOVA due to flexibility of the model should

circumstances become more complex and due to the presence of slightly unequal group sizes, which is a violation of ANOVA assumptions. Where justified, additional t-tests were conducted to supplement regression analyses. All analyses were conducted using IBM SPSS 19.0 statistical software.

RESULTS

OF

Locomotor and anxiety-like behavioral variables were analyzed using simple regressions for both HA and LA groups of animals. The regression was calculated by setting each drug group against saline to test for statistical significance. This allows a predictive equation to be calculated so that $y = b_0 + b(\text{drug group}) + \text{error}$. However, where warranted, additional t-tests were used to determine differences between HA and LA animals and between drug groups other drug groups of interest. Three variables were considered, total distance traveled in the arena, distance traveled in the center of the arena, and time spent in the center of the arena.

The main locomotor variable assessed was the total distance traveled in the OF arena. This variable was measured in 3 x five minute intervals over the 15 minute test. Regression output for the total distance traveled variable is summarized in Tables 3 and 4. Initially, each drug group, baclofen (baclofen +saline), nicotine (saline + nicotine), and baclofen + nicotine, was compared to the saline group to determine statistical differences. Each 5 minute interval was analyzed independently of the others. In the HA group, after 5 minutes, there was a statistically significant difference in the total distance

traveled variable for each of the three drug groups when compared to saline animals, $p <$ 0.05 (see Figure 1).

Table 3. Total distance traveled by 5 minute intervals in HA animals. Means on the graph are represented by $y = b_0 + b$ (drug group). In this condition, all drug groups were compared against saline for statistical significance. At 5 minutes, $R^2 = .706$, at 10 minutes, $R^2 = .377$, and at 15 minutes, $R^2 = .238$.

Drug Group/Time Interval	B	SE B	Significance
Constant (B_o)/ @ 5 minutes	1552.955	77.370	.001
Baclofen + Nicotine/@ 5 minutes	-1009.275	112.119	.001
Baclofen/@ 5 minutes	-309.835	112.119	.009
Nicotine/@ 5 minutes	-571.977	115.336	.001
Constant (B_o)/ @ 10 minutes	978.855	64.883	.001
Baclofen + Nicotine/@ 10 minutes	-359.995	94.024	.001
Baclofen/@ 10 minutes	-358.115	94.024	.001
Nicotine/@ 10 minutes	-115.577	96.722	.240
Constant (B_o)/ @ 15 minutes	643.327	58.369	.001
Baclofen + Nicotine/@ 15 minutes	-159.477	84.585	.067
Baclofen/@ 15 minutes	-127.007	84.585	.142

Nicotine/@ 15 minutes	104.862	87.012	.236

Table 4. Total distance traveled by 5 minute intervals in LA animals. Means on the graph are represented by $y = b_0 + b$ (drug group). In this condition, all animals were compared against saline for statistical significance. At 5 minutes, $R^2 = .434$, at 10 minutes, $R^2 = .191$, and at 15 minutes, $R^2 = .207$.

Drug Group/Time Interval	B	SE B	Significance
Constant (B_o)/@ 5 minutes	1473.878	109.442	.001
Baclofen + Nicotine/@ 5 minutes	-759.428	150.856	.001
Baclofen/@ 5 minutes	-240.998	150.856	.119
Nicotine/@ 5 minutes	-357.598	150.856	.023
Constant (B_o)/ @ 10 minutes	957.700	94.560	.001
Baclofen + Nicotine/@ 10 minutes	-232.140	130.342	.084
Baclofen/@ 10 minutes	-222.750	130.342	.096
Nicotine/@ 10 minutes	58.080	130.342	.659
Constant (B_o)/@ 15 minutes	756.267	99.971	.001
Baclofen + Nicotine/@ 15 minutes	-236.367	137.801	.095
Baclofen/@ 15 minutes	-180.567	137.801	.199
Nicotine/@ 15 minutes	124.843	137.801	.371

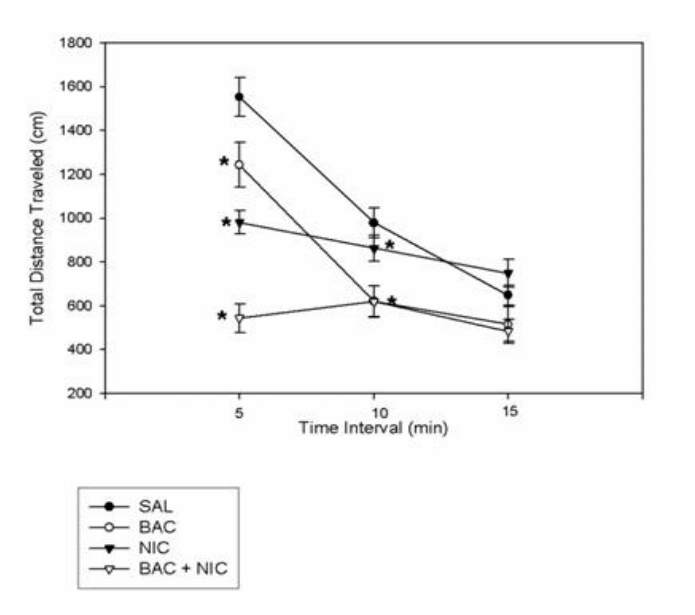

Figure 1. Means of all drug conditions, saline + saline (SAL), baclofen + saline (BAC), saline + nicotine (NIC), and baclofen + nicotine (BAC + NIC), for the total distance traveled variable at time intervals of 5 minutes, 10 minutes, and 15 minutes in HA animals. * = a significant difference between drug group and saline, $p < .05$.

At the 10 minute interval, both the baclofen and baclofen + nicotine groups showed statistically significant differences compared to saline ($p < .05$), but nicotine did not. By the 15 minute interval, none of the drug groups showed significant differences with respect to saline. However, at the 5 minute timepoint, there was a significant difference between the baclofen and baclofen + nicotine groups, $t(18) = 5.786$, $p < .001$. And, at all three time points, there were significant differences between nicotine and baclofen + nicotine groups among HA animals, $t(17) = 5.097$, 2.613, and 3.197 respectively, $p < .05$ (see Figure 2).

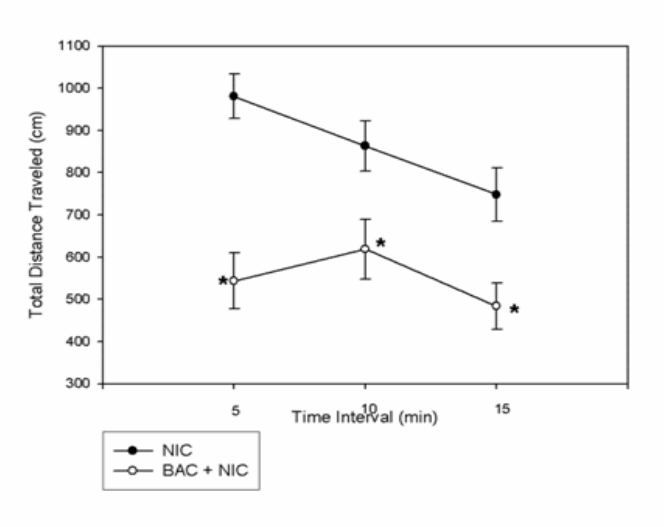

Figure 2. Means of the saline + nicotine (NIC) and baclofen + nicotine (BAC + NIC) groups for the total distance traveled variable at time intervals of 5, 10, and 15 minutes in HA animals. *$p < .05$

Among LA animals, there were also statistical differences between drug groups on the total distance traveled variable (see Figure 3). There were statistically significant differences between saline animals and the animals that received either nicotine or baclofen + nicotine in the first 5 minutes, $p < .05$. By 10 minutes and 15 minutes, these differences were no longer significant. There were also statistically significant differences on the total distance traveled variable among other drug groups in LA animals. In the first 5 minutes there was a significant difference between baclofen and baclofen + nicotine, $t(18) = 3.317$, $p < .01$. There were also significant differences between nicotine and baclofen + nicotine groups at 5, 10, and 15 minutes, $t(18) = 3.144$, 3.488, 3.552 respectively, $p < .01$ (see Figure 4).

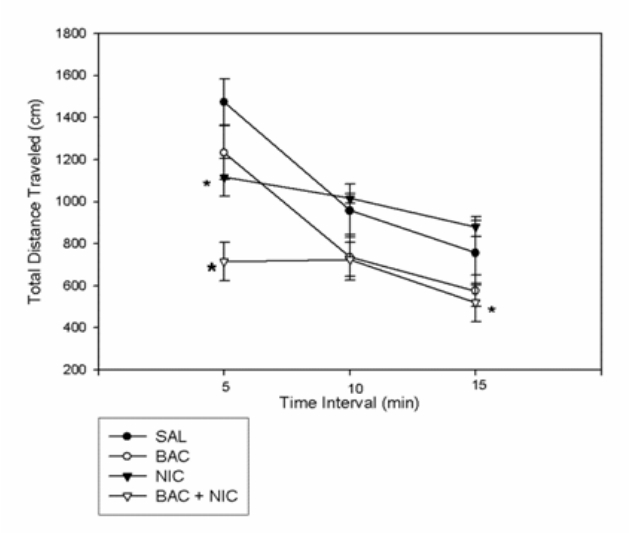

Figure 3. Means of all drug conditions, saline + saline (SAL), baclofen + saline (BAC), saline + nicotine (NIC), and baclofen + nicotine (BAC + NIC), for the total distance traveled variable at time intervals of 5 minutes, 10 minutes, and 15 minutes in LA animals. * = a significant difference between drug group and saline, $p < .05$.

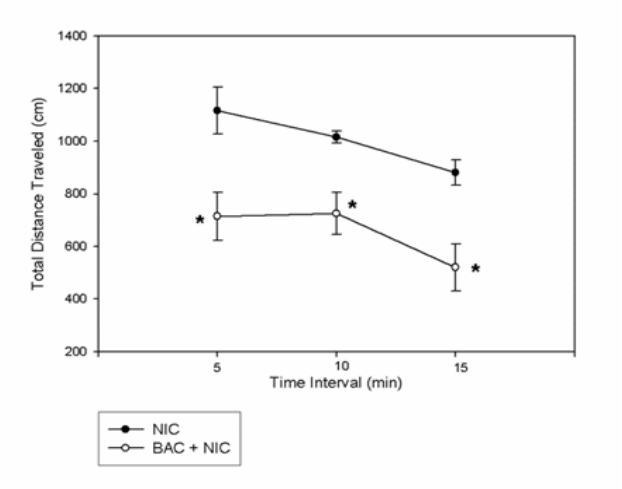

Figure 4. Means of the saline + nicotine (NIC) and baclofen + nicotine (BAC + NIC) groups for the total distance traveled variable at time intervals of 5, 10, and 15 minutes in LA animals. Statistically significant differences occurred at all three time points. +$p <$ .01

While not many substantial differences existed between HA and LA animals in locomotor behavior, one notable difference was in the effect of baclofen on the total distance traveled variable. Among HA animals, baclofen animals were statistically significantly different from saline animals on the total distance traveled variable at the 5 minute, $t(18) = -2.763$, $p < .01$, and at the 10 minute intervals, $t(18) = -3.809$, $p \leq .001$ (see Figure 5). In LA animals, there are no significant differences between baclofen and saline animals (see Figure 6).

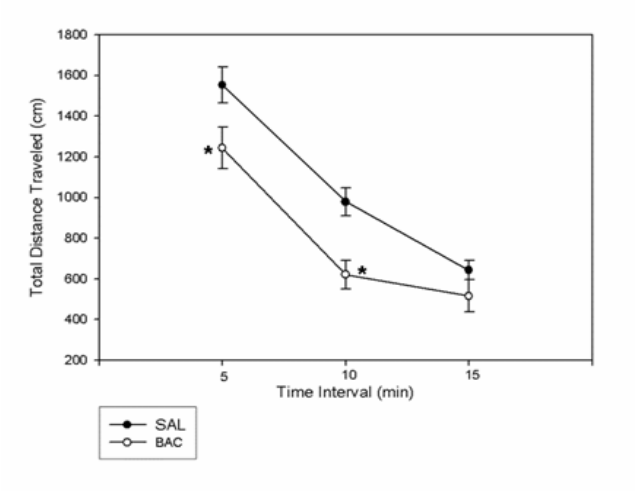

Figure 5. Means of the baclofen + saline (BAC) and saline + saline (SAL) groups for the total distance traveled variable at time intervals of 5, 10, and 15 minutes in HA animals. Statistically significant differences existed at the 5 and 10 minute intervals. *$p < .01$

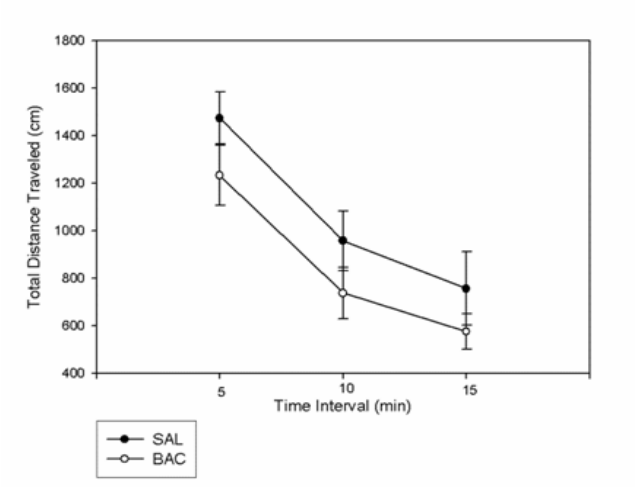

Figure 6. Means of the baclofen + saline (BAC) and saline + saline (SAL) groups for the total distance traveled variable at time intervals of 5, 10, and 15 minutes in LA animals. No statistically significant differences existed.

The second variable measured was distance traveled in the center. Once again, this variable was assessed at 5, 10, and 15 minute intervals. Distance traveled in the center was linked to both locomotor and anxiety-like behavior. Among HA animals, only one comparison showed statistical significance on this variable. When compared to saline animals, nicotine animals were statistically significantly different at the 15 minute interval of distance traveled in the center of the arena, $t(19) = 2.170$, $p < .05$ (see Figure 7). Among LA animals, there were no significant differences on this variable (see Figure 8).

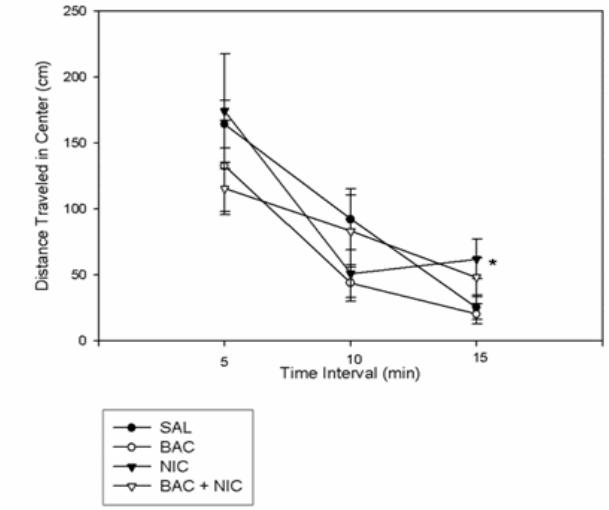

Figure 7. Means of all drug conditions, saline + saline (SAL), baclofen + saline (BAC), saline + nicotine (NIC), and baclofen + nicotine (BAC + NIC), for the distance traveled in the center variable at 5, 10, and 15 minute intervals in HA animals. Only the comparison between SAL and NIC at 15 minutes was significantly different. *$p < .05$

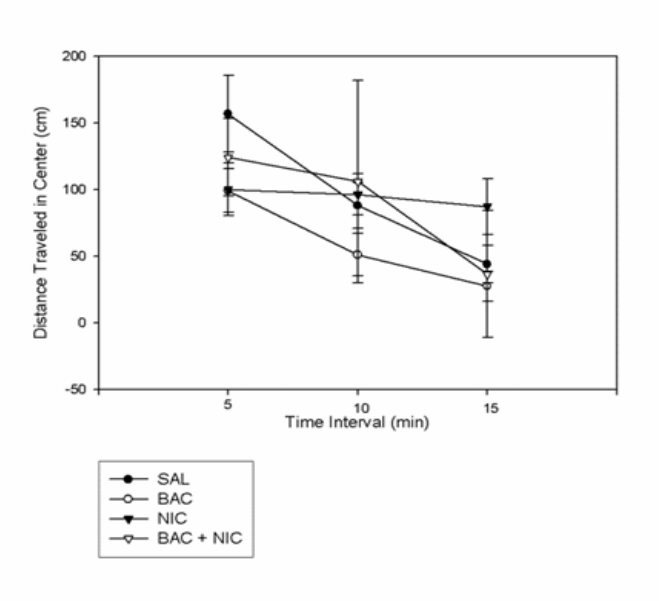

Figure 8. Means of all drug conditions, saline + saline (SAL), baclofen + saline (BAC), saline + nicotine (NIC), and baclofen + nicotine (BAC + NIC), for the distance traveled in the center variable at 5, 10, and 15 minutes in LA animals. There were no significantly different relationships.

The third variable evaluated was time spent in the center of the arena, a variable used to gauge anxiety-like behavior. Again, this variable was measured at 5, 10, and 15 minutes. Almost all of the statistically significant comparisons involved the baclofen + nicotine drug groups. Among HA animals, there was a significant difference between the animals that received baclofen + nicotine and those that received saline on time spent in the center at 5 minutes and 15 minutes, $t(19) = 2.413$ and 2.275 respectively, $p < .05$ (see Figure 9). Additionally, there was a significant difference between the baclofen and baclofen + nicotine groups on time spent in the center at the 5 minute mark, $t(18) = -2.235$, $p < .05$. Among LA animals, there was a significant difference between baclofen + nicotine and saline animals on time spent in the center at 5 and 10 minutes, $t(19) =$

2.430 and 2.452 respectively, $p < .05$, and between nicotine and saline animals at 15 minutes, t(19) = 2.236, $p < .05$ (see Figure 10). In addition, there were significant differences between the baclofen and baclofen + nicotine animals at 5, t (18) = -2.299, $p < .05$, and 10 minutes, t(18) = -3.061, $p < .01$.

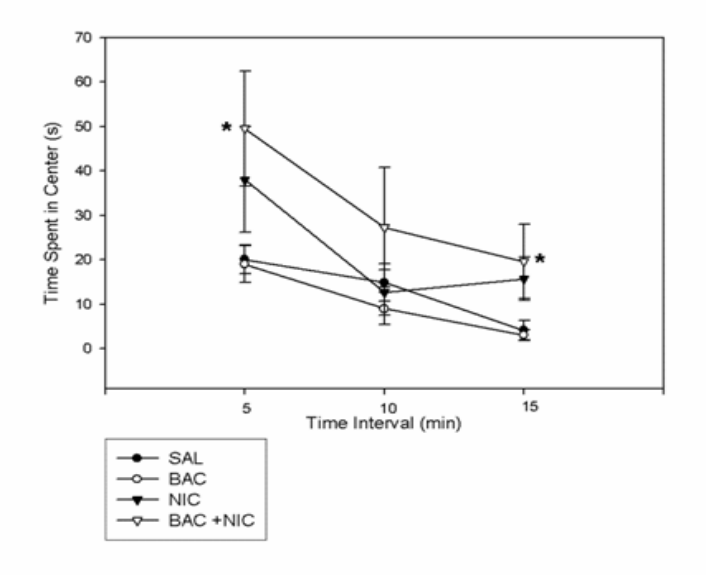

Figure 9. Means of all drug conditions, saline + saline (SAL), baclofen + saline (BAC), saline + nicotine (NIC), and baclofen + nicotine (BAC + NIC), on center time at 5, 10, and 15 minutes in HA animals. There was a statistically significant difference between BAC + NIC and SAL at 5 and 15 minutes, $^{*}p < .05$.

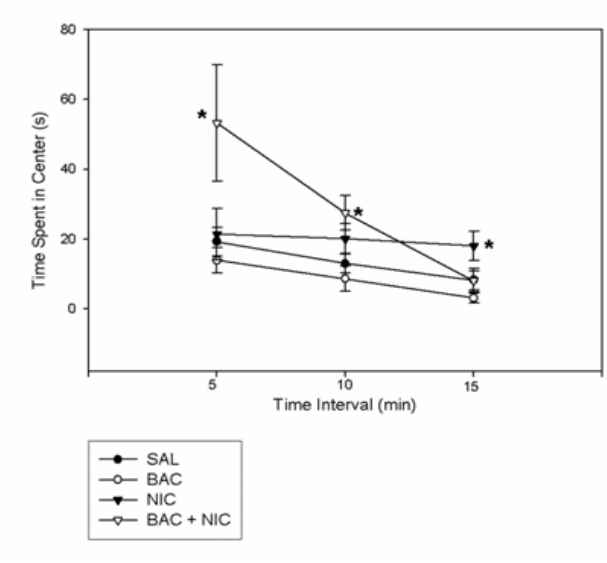

Figure 10. Means of all drug conditions, saline + saline (SAL), baclofen + saline (BAC), saline + nicotine (NIC), and baclofen + nicotine (BAC + NIC), on center time at 5, 10, and 15 minutes in LA animals. There were significant differences between BAC + NIC and SAL animals at 5 and 10 minutes, and between NIC and SAL at 15 minutes. $^{*}p < .05$

CPP

Linear regressions were analyzed comparing each drug condition to saline on the variable of difference score. When a preliminary t-test was run comparing nicotine to saline using the sample as a homogenous group, there was no statistically significant distinction between nicotine and saline, t(35) = -1.320, $p = .196$, suggesting that CPP training had not been successful. However, when the sample was divided into HA and LA groups, this concept was no longer the case.

Among HA animals there were statistically significant variations between animals administered nicotine and baclofen + nicotine and animals administered saline. Regression output is summarized in Table 5.

Table 5. Comparisons between drug groups and saline on the variable of difference score in HA animals. Means on the graph are represented by $y = b_0 + b$ (drug group). $R^2 = .294$

Drug Condition	B	SE B	Significance
Constant (B_0)	3.125	26.458	.907
Baclofen + Nicotine	98.275	35.497	.009
Baclofen	18.653	36.362	.611
Nicotine	102.319	36.362	.008

Both the nicotine and baclofen + nicotine groups had a significantly higher difference score than the saline group (see Figure 11).

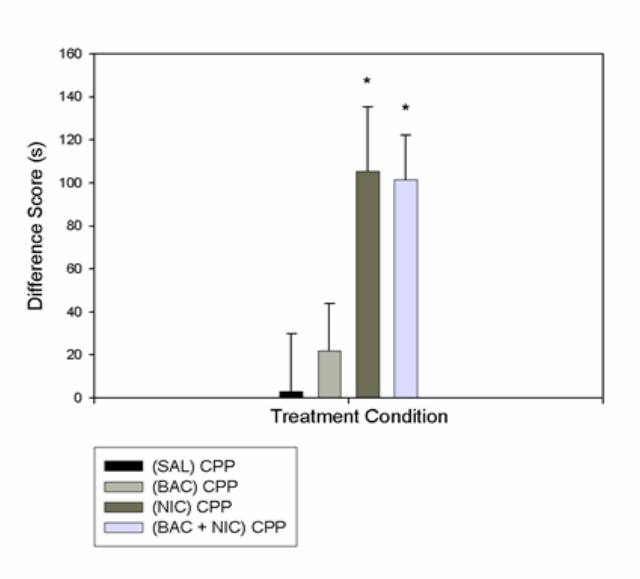

Figure 11. Means of all drug conditions, (saline + saline)CPP [(SAL)CPP], (baclofen + saline)CPP [(BAC)CPP], (saline + nicotine)CPP [(NIC)CPP], and (baclofen + nicotine)CPP [(BAC + NIC)CPP], on the variable of difference score in HA animals. Significant differences existed between (SAL)CPP animals and both (NIC)CPP and (BAC + NIC)CPP animals. *$p < .01$

Among LA animals, there were no significant distinctions between the saline group and any of the drug groups (see Figure 12). Regression output is summarized in Table 6.

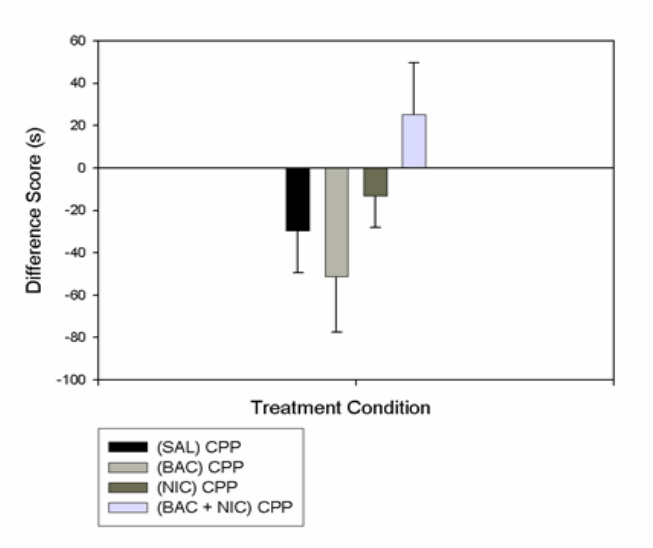

Figure 12. Means of all drug conditions, (saline + saline)CPP [(SAL)CPP], (baclofen + saline)CPP [(BAC)CPP], (saline + nicotine)CPP [(NIC)CPP], and (baclofen + nicotine)CPP [(BAC + NIC)CPP], on the variable of difference score in LA animals. There were no significant differences between saline and any drug group.

Table 6. Comparisons between drug groups and saline on the variable of difference score. Means on the graph are represented by $y = b_0 + b$ (drug group). $R^2 = .158$

Drug Condition	B	SE B	Significance
Constant (B_0)	-29.667	22.898	.204
Baclofen + Nicotine	54.867	31.563	.091
Baclofen	-21.433	31.563	.502
Nicotine	16.367	31.563	.607

DISCUSSION

The present study examined the impact individual differences in anxiety-like behavior had on locomotion, anxiety-like behavior, and reward after administration of nicotine and baclofen in adolescent rats. Adolescent Sprague-Dawley rats were separated into HA and LA groups using a median split analysis based on time spent in the white chamber of a biased CPP chamber. Subsequent testing using OF found some notable differences in innate anxiety-like behavior and later locomotor and anxiety-like behavior in rats dependent on HA/LA status and drug administered. Notably, baclofen administration significantly decreased locomotor behavior from saline levels in HA animals, but did not do so in LA animals. In both HA and LA groups, baclofen and nicotine co-administration significantly decreased locomotor behavior from locomotor activity levels in animals administered nicotine alone. The open field was also used to examine potential differences in anxiety-like behavior. Baclofen administration failed to produce differences in anxiety-like behavior between HA and LA groups, but nicotine administration and baclofen + nicotine co-administration had a slightly more profound effect on anxiety-like behavior in LA than HA animals. Additionally, in single-trial nicotine CPP testing, only HA rats formed CPP to nicotine and baclofen + nicotine administration. LA rats failed to form CPP to any drugs tested.

Rats were assigned to HA or LA groups using performance in the CPP chamber, either prior to OF testing, or data from the pretest day of CPP testing. This method was utilized because pilot testing found that pretesting with elevated plus maze (EPM) prevented adolescents from forming single-trial nicotine CPP (unpublished pilot data). However, the biased CPP chamber employed is highly similar to the light-dark box, so that it is likely that it acts as a viable measure of anxiety-like behavior. This method also has the benefit of reducing number of testing days in rats, as the window of time to obtain single-trial nicotine CPP is very narrow, approximately P28-P32 in Sprague Dawleys (Belluzzi, et al., 2004; Brielmaier, et al., 2007; Brielmaier, et al., 2008). This methodology also alleviates the issues of EPM blocking the ability to achieve single-trial nicotine CPP and the inability to use OF as a pretest due to its inclusion later in the protocol.

One particularly notable finding of this study is that HA rats show a statistically significant difference between those administered baclofen + saline and controls (saline + saline) [see Figure 5]. This relationship is no longer statistically significant in the LA group (see Figure 6). Administration of baclofen is known to sedate locomotion in rats, though often at higher doses (Le Foll, et al., 2008; Frankowska, et al., 2009; Palmatier & Bevins, 2002). This study is of note in that adolescents may be slightly more susceptible to the sedating effects of baclofen, even though the R (+) baclofen enantiomer is used here and is relatively more active; 0.6 mg/kg is a lower dose than used in other literature and pretest data in adults showed that this dose had no sedating effects (unpublished pretest data). In addition, it is of interest, that at least in adolescents, innate anxiety-like behavior is a variable that determines reaction to the locomotor effects of baclofen. It is

entirely possible that this is due to the systemic action of the $GABA_B$ agonist activating receptors which play a role in both anxiety-like behavior and locomotion (Amikishieva & Semendyaeva, 2007; Bowery, 2006; Mombereau, et al., 2004). It is also possible that these findings are only applicable to adolescents as adolescents are known to exhibit higher levels of anxiety-like behavior than adults in numerous paradigms (Lynn & Brown, 2010); clearly these results would have to be replicated in adults.

Another noticeable finding is that in both HA and LA groups, dosing with baclofen + nicotine significantly reduced the locomotor behavior in comparison to rats dosed only with nicotine (see Figures 2 and 4). This is supported by literature which shows that baclofen reduces the activity levels of adult rodents dosed with nicotine (Lobina, et al., 2011; Palmatier & Bevins, 2002) and cocaine (Frankowska, et al., 2009). The means of rats dosed with nicotine and rats dosed with baclofen + nicotine are significantly lower than saline-dosed rats at several time points. It appears that the $GABA_B$ activation in the rats dosed with baclofen + nicotine is playing a role in the further suppression of locomotor activity due to the fact that the addition of baclofen suppresses locomotor activity further than in nicotine dosed rats.

It was expected that dosing with baclofen and baclofen + nicotine would have a more significant impact on anxiety-like behavior. This hypothesis was driven by several studies suggesting a link for the $GABA_B$ receptor in anxiety-like behavior. Genetic work has shown that $GABA_{B(1)}$ -/- mice, which lack functional $GABA_{B(1)}$ receptors, were more anxious than wildtype littermates in the light-dark box and staircase test (Mombereau, et al., 2004). Studies with baclofen have shown that baclofen administration has anxiolytic

effects on the EPM in male mice (Amikishieva & Semendyaeva, 2007), however, that baclofen administration was unable to alter the dose-dependent anxiety-like behavior produced by nicotine in male mice (Varani & Balerio, 2012). It is possible that baclofen has little effect on anxiety-like behavior in this study because the subjects are adolescents, whereas most work has been done with adults. Though GABA is the main inhibitory neurotransmitter in adults, it actually serves as an excitatory neurotransmitter in early postnatal development (Ben-Ari, Khazipov, Leinekugel, Caillard, & Gaiarsa, 1997) and studies have shown that during early adolescence, GABA neurons respond more weakly to GABA agonists due to immaturity of the neurons (Cohen, Lin, & Coulter, 2000). It is, therefore, entirely possible that these findings would not be replicated in adults. This is also supported by the body of work delineating the increased vulnerability of the adolescent to nicotine (see O'Dell & Khroyan, 2009). However, this hypothesis is in opposition to this study's findings that adolescents could show profound locomotor effects to baclofen. There is a possibility that the GABA neurons in the movement areas of the brain are maturing more quickly than areas associated with anxiety-like behavior, but at this moment, this question does not seem to have been examined.

The present study also found that HA rats that were dosed with either nicotine or baclofen + nicotine were able to form single-trial CPP, while no group among LA rats were able to achieve CPP. This suggests that high anxiety-like behavior plays a role in nicotine CPP and that using the pretest day is a valid measure of naïve anxiety-like behavior. Previous work with cocaine has suggested that high anxiety rats achieve higher

rates of CPP (Pelloux, et al., 2009), in addition to higher rates of cocaine self-administration (Dilleen, et al., 2012; Schramm-Sapyta, et al., 2011), though this relationship has not been seen with alcohol (Langen & Fink, 2004). Among the HA rats, it seems likely that nicotine is driving the CPP effect among rats dosed with baclofen + nicotine, as there was no significant alterations in the difference scores between the nicotine and baclofen + nicotine groups. However, it is noteworthy that baclofen co-administration did nothing to alter nicotine CPP. Previous studies using baclofen have shown that administration of 3 mg/kg of R (+) baclofen, though neither 0.3 or 1 mg/kg of baclofen blocked nicotine CPP (Le Foll, et al., 2008). In addition, administration of baclofen was capable of preventing reinstatement of nicotine CPP in mice (Fattore, et al., 2009). It may be that baclofen has an ability to block nicotine CPP, but only at high doses. However, it would seem that, at least in adolescents, the sedative effects at such a high dose may be problematic. The administration of baclofen did not have a long-lasting locomotor impact that impaired CPP, as is demonstrated by the fact the baclofen + nicotine group acquired single-trial nicotine CPP at roughly the same rate as nicotine rats. It is also noteworthy, that during OF testing, nicotine dosing did not alter anxiety-like behavior in either HA or LA groups, supporting the concept that the anxiety-like behavioral difference here is innate and not drug-induced.

This study has areas that are worth expanding on. Since previous work has found higher doses of baclofen effective in attenuating nicotine CPP, it may be worthwhile to examine if adolescents can be treated at the higher dose without severe locomotor sedation. However, at this point, it does appear that baclofen, while it may be useful as a

treatment in adults, is not an option as a preventative or blocking agent of nicotine reward in adolescents. In addition, it is may also be beneficial to apply the anxiety aspect of this work to adults to see if anxiety status can select out adults that will form nicotine CPP over multiple conditioning sessions.

In summary, testing using OF found some notable differences in innate anxiety-like behavior and later locomotor and anxiety-like behavior in rats dependent on HA/LA status and drug administered. Notably, baclofen administration significantly decreased locomotor behavior from saline levels in HA animals, but did not do so in LA animals. In both HA and LA groups, baclofen and nicotine co-administration significantly decreased locomotor behavior from locomotor activity levels in animals administered nicotine alone. The open field was also used to examine potential differences in anxiety-like behavior. Baclofen administration failed to produce differences in anxiety-like behavior between HA and LA groups, but nicotine administration and baclofen + nicotine co-administration had a slightly more profound effect on anxiety-like behavior in LA than HA animals. In addition, the dose of baclofen used had no effect on single trial nicotine CPP in adolescents, but anxiety status emerged as a predictor of which rats would form CPP. Therefore, this study does not lend support to the use of baclofen as a treatment for nicotine addiction, but elucidates the coexistence of adolescence and high anxiety as dual roles in forming nicotine reward.

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CURRICULUM VITAE

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