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Honors Thesis

The effects of nicotine on responsivity to repeated social stress in early adolescent, late adolescent, and young adult male rats

Kathryn Dean

U58515916

Thesis Director- Cheryl L. Kirstein, Ph.D.

Committee Member- Antoniette M. Maldonado-Devincci, Ph.D.

Abstract

Nicotine is the addictive compound in tobacco and use of this drug, primarily through cigarette smoking, is commonly initiated during adolescence. Human adolescents use nicotine to reduce stress and anxiety. Once adolescents begin smoking, increased aggressiveness has been observed in males in response to social stress. In the present work, nicotine (0.6 mg/kg s.c.) or saline was administered to experimental animals and reactivity to repeated social stress was assessed. Beginning on PND 31 for early adolescents, PND 41 for late adolescents and PND 60 for young adults, animals were administered nicotine or saline, and immediately after drug administration, an intruder was introduced to the home cage of the experimental animal for ten minutes to induce social stress. This procedure was repeated over five days. Each ten-minute social stress exposure was video recorded and quantified offline for changes in aggressive behaviors in experimental animals, characterized as latency and duration to supine position and attack frequency. The experimental animal was also quantified for pinning frequency. Nicotine initially depressed general activity levels and it subsequently increased across days in an age-dependent manner, with the greatest increase for the early adolescent animals and the least in young adult animals. Results indicate that nicotine treatment decreased aggressive behavior in all age groups, relative to saline-treated rats. This decrease was age dependent, as supported by the observation of the greatest latency, shortest duration, lowest frequency of pins and fewest attacks to the intruder in the young adult nicotine-treated rats. The degree of depression was less for the late adolescent animals than the young adult animals, and the early adolescent animals exhibited the least depression in aggressive responses and activity level. Together, these data indicate reactivity to repeated nicotine administration and social stress is age-dependent and is readily

depressed by drug treatment. Future work should assess dose-dependent alterations in social stress reactivity.

Introduction

Adolescence is the peak period in which drug addiction becomes a method of coping and addiction often progresses from legal to illegal drugs (Bruns & Geist, 1984). According to the Surgeon General's 1988 report, nicotine is the drug in tobacco that causes addiction, and nearly all smokers meet the diagnostic criterion for being dependent (Dept. of Health and Human Services, 1988). In their 1984 study, Bruns and Geist found that nicotine was the most common entry drug. Adolescents first try beer and wine and cigarettes, which contain tobacco, and those that seek more intense drugs usually transition from cigarettes, beer and wine to hard liquor and eventually to marijuana and other illegal drugs. The role of nicotine as one of the gateway drugs into adolescent drug use makes it an extremely important drug to study. Once the causes and effects of cigarette use in adolescence are understood, more effective methods of prevention can be put in place, decreasing not only the use of nicotine, but also the use of the other drugs, legal and illegal, that often follow the use of cigarettes. Studies conducted by Kandel in 1975 and Kandel and colleagues in 1992 and 1994 showed that nicotine exposure during adolescence leads to an increased probability of nicotine and other drug use in adulthood, possibly because it causes alterations to the mesoaccumbens dopamine system that causes individuals to not only seek out nicotine, but maintain use. According to the US Department of Health and Human Services (1994), The Campaign for Tobacco-Free Kids and the Center for Disease Control and Prevention, of the adult smokers in the United States, 80% began smoking before the age of 18, and adolescent smokeless tobacco users are more likely than nonusers to become cigarette smokers in adulthood. In 2009, the Substance Abuse and Mental Health Services Administration found that each day, approximately 850 adolescents become regular cigarette smokers- defined as an adolescent that smokes an average of seven cigarettes per day (Gilliland et al., 2006). Also

in 2009, the CDC found that 17.2% of high school students smoke cigarettes, 10.9% of high school students smoke cigars, and 6.7% of high school students use smokeless tobacco. For middle school students, the findings were 5.2%, 3.9%, and 2.6 % respectively. In each of the six categories, the percentage of users was higher for males than females (CDC, 2010). Despite these alarming statistics, cigarette use among eighth, tenth, and twelfth grade students has decreased overall in the past ten years (Johnston et al., 2010). Still though, among 8th, 10th, and 12th graders, disapproval of smoking, acknowledgment of the risks associated with smoking, and the number of students that see smoking as a negative behavior has leveled off in recent years (Johnston et al., 2010). Therefore, the need to understand the effects of cigarettes and nicotine in particular, specifically in adolescents, becomes increasingly important. In order to affect a decrease in cigarette/nicotine use among high school students, the behavioral and physiological effects of nicotine on the adolescent brain must continue to be studied.

Nicotine use, through tobacco, is more prevalent among adolescent males than females in both the high school and middle school age group (CDC, 2010). It is also more common among adolescents who are stressed than those who are not (Bruns & Geist, 1984). Therefore, it is important to determine how nicotine affects responsivity to social stressors in early adolescents, late adolescents, and young adults, which can be modeled using a rodent model, so the findings can be extrapolated to human subjects. Cigarette use, specifically during adolescence, is often associated with life stress, most often caused by negative life events. Negative affect is related to an increase in smoking, and the relationship between stress and smoking is similar for females and males (Wills et al, 2002). In Bruns and Geist's 1984 study, it was determined that high previous and current life stress scores were associated with drug abusers and poly-drug users, and that common stressors for high school students include body image, approaching adulthood,

and increased awareness of life demands. Nicotine is a mood-altering drug that is used by adolescents to reduce stress and anxiety and also to diminish boredom (DiFranza et al, 2002). Of the adolescent smokers in the United States, 70% smoke cigarettes in response to social stressors (DiFranza et al, 2002). This indicates there is a direct relationship between cigarette smoking and social stress in adolescents. Once adolescents begin smoking, the response to stressors is thought to change as a result of the addictive properties of nicotine. The goal of the proposed experiment is to monitor these changes, in early adolescent, late adolescent, and young adult male rats, so that the findings can then be extrapolated and related to similar phenomena in human adolescents.

Animal models are often used in scientific studies, specifically those involving behavior and addiction. Rats share many physiological similarities with humans, and this, combined with the large size (Chan & Agca, 2008), short gestation-time, numerous offspring, and shorter life-span, make them ideal models for various studies. In this study, Sprague Dawley rats, characterized as being calm and easy to handle, were used. This breed of rat also reproduces efficiently- becoming sexually mature at 65 days of age. Responsivity to nicotine was measured by monitoring changes in aggressive activity among animals exposed to social stress, treated with nicotine or a saline served as a vehicle for control animals, over a five day period. In human subjects, sensitization, often characterized as an increase in activity level, is hard to observe because it is unclear what areas of the brain are affected. In rats, it is often measured by monitoring changes in locomotor activity (DiFranza & Wellman, 2007), further supporting the idea that the Sprague Dawley rat is an ideal animal model, because the effects and behaviors under investigation can be easily observed.

Upon initial intake of nicotine, a depressant effect is often observed, but after a few treatments, the effects change from that of a depressant to a stimulant (Clark & Kumar 1983b). In rats, acute systemic nicotine administration causes locomotor activation, and this may be preceded by a brief depression of locomotor activity (Clarke & Kumar, 1983a). Repeated injections causes a build-up of tolerance to the depressant effect (Collins et al, 1988) and sensitization to the stimulant effect (Clarke and Kumar, 1983a; Ksir et al., 1985). Sensitization is often thought of as reverse tolerance, and occurs when repeated exposures produce responses greater than those initially observed (DiFranza & Wellman, 2007). Studies are still being conducted in order to determine the mechanism of the occurrence of sensitization. It is known, however, that nicotine stimulates dopaminergic neurons in the ventral tegmental area, causing a release of dopamine in the nucleus accumbens septi (Balfour et al., 1998). Sensitization lowers the dose of nicotine that is required to cause the rapid burst pattern of dopamine overflow into the nucleus accumbens septi (Nisell et al., 1996). With nicotine, it is observed through increased locomotor activity and reaches a peak 5-7 days after the animal was initially exposed (DiFranza & Wellman, 2007). Sensitization is hypothesized to contribute to the escalation of drug use from casual to abusive. In previous work, rats worked to obtain nicotine in a self-administration paradigm, which is used habitually over long time periods, so the potential for long-term harmful effects increases and sensitization may be one of the mechanisms mediating the change in behavior (Vezina et al, 2007).

The predominant focus of past studies has been to determine if sensitization occurs in female and male adolescent and adult rats, the degree of sensitization- mostly through measurements of change in locomotor activity, and if the sensitization experienced in adolescents persists into adulthood. Little research has been done on the relationship between

changes in aggressive behavior in adolescent and adult male rats that are treated with nicotine and subsequently stressed. In a study conducted by File and colleagues, it was observed that nicotine enhances aggressive behavior in stressed human male adolescents, while nicotine induces a calming effect in stressed females (File, et al., 2001). For this double-blind study, sixteen male and sixteen female students were assigned to either a nicotine group or a placebo group. Tests to measure cognitive abilities were administered, and it was determined that nicotine use does not affect cognitive abilities. Mood and aggression rating scales were completed before testing, participants were asked to complete a series of stressful cognitive tests and then mood and aggression changes were analyzed. Nicotine blocked the stress induced mood changes in females, while enhancing the feelings of being less calm, discontented, quarrelsome, hostile, rebellious, and angry in males (File et al., 2001). In this study, participants were tested one time, and age was not a factor.

In the present study, male rats in early adolescence, late adolescence, and young adulthood are administered nicotine (0.6mg/kg) or saline, and then immediately exposed to social stress. Sensitization, discussed above, was expected to occur, and often manifests through changes in activity level. For this study, it is monitored specifically as changes in aggressive activity- determined by monitoring the time of latency to supine position for the experimental animal, the amount of time that the experimental animal is in the supine position, the number of pins (intruder to experimental subjects) and number of attacks (experimental animal to intruder), and non-aggressive activity- determined by monitoring the time in ambulation of the experimental animal. Testing was conducted for five consecutive days, in order to assess whether an increase or decrease in aggressive and general activity occurred during that time in an

age-dependent and drug-dependent manner. Male rats of three different age groups are tested so that any variation in responsivity with age is detected.

Methods

A total of 60 male rats (Sprague-Dawley), were used for this study. Rats were offspring of established breeding pairs, and were sexed and culled, 10 pups per litter, on postnatal day (PND) 1. They were weaned and housed in groups of three on PND 21 in a humidity and temperature controlled vivarium for a 12:12 hour light/dark cycle (0700/1900h). No more than 1 male pup per litter was used in a given condition and animals were cared for as directed by the NIH. The present experiment was conducted using video-taped sessions that were scored offline for the behavioral changes induced as described above across five consecutive days in early adolescent, late adolescent and young adult male rats.

On PND 29 for early adolescents, PND 39 for late adolescents, and PND 58 for young adults, animals were individually housed. On PND 31 (early adolescent), PND 41 (late adolescent), and PND 60 (young adult), animals were removed from the colony, weighed, injected with either nicotine (0.6mg/kg), for experimental animals or saline as a vehicle in control animals and placed in the home cage immediately following the injection. An intruder rat (100-200g > resident; i.e. experimental animal) was placed in the home cage of the experimental subject for a 10 minute session that was video recorded and quantified offline. Immediately after the ten minute session, the intruder was removed and the experimental animal was placed in a clean cage and returned to the colony room. The following day, the experimental animal was weighed and injected with either nicotine (experimental;) or saline (vehicle), and placed into the same home cage from the previous day. An intruder rat that had not previously been paired with the experimental animal was placed in the cage, and the session was video recorded. This procedure was repeated for PND 31-35(early adolescent), 41-45(late adolescent), and 60-64 (young adult).

Following the sessions, videos were analyzed for five factors. The first was the time the experimental animal spent in ambulation. The next was the time of latency to supine position, or the amount of time that passed before the intruder rat pinned the experimental rat on its back. The time spent in the supine position, or the amount of time needed for the experimental rat to free and right itself was measured. The number of pins (intruder to experimental subject) was recorded so that the average amount of time in supine position per pin could be found. The number of attacks (experimental animal to intruder) was recorded as another means of monitoring aggression and the changes that occur within each age group and treatment over a five day period. A summary of the methods is provided in Figure 1. An intruder rat was placed in the cage of the experimental rat in order to serve as a social stressor, modeling the social stressors that adolescent humans face. Rats of three different age groups were tested so that variation in the response to social stress with age could be monitored. The use of age groups that are close, but different, serve to aid in determining, more precisely, when the observed effects begin and change. The behaviors being monitored were specifically chosen because they represent aggressive interaction between the experimental and intruder rat as well as general activity. A more aggressive rat will attack more, and spend less time in the supine position, and scoring the videos for these activities will aid in determining how aggressive behavior changes with age and nicotine administration. A more active animal will spend more time in ambulation. The collected data was analyzed using a 3 factor mixed-model design ANOVA for Age (3; 35, 45, 64), Drug (2; nicotine, saline) and day as repeated measure.

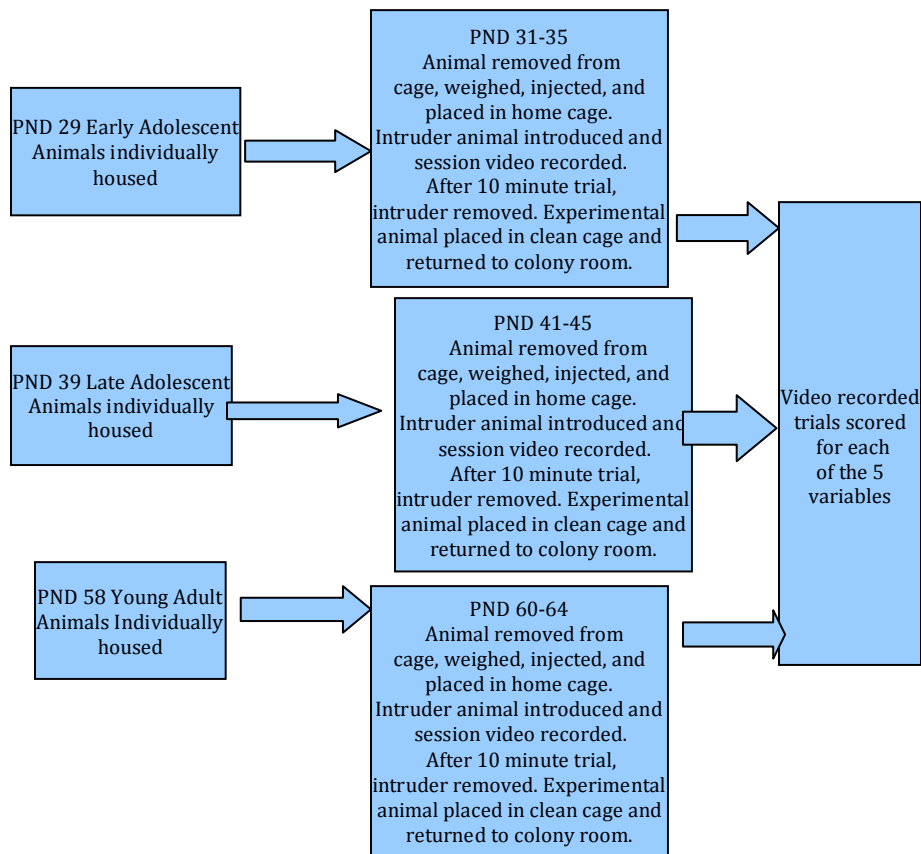


Figure 1 Contains the overview of the procedure that was followed for this study.

Results

Data were initially analyzed using a 3 factor mixed- model design ANOVA for Age (3; PND 35, PND 45, PND 64), Drug (2; nicotine, saline) and Day for 1) ambulation of the experimental animal, 2) time of latency to supine, 3) time in supine, 4) pin frequency 5) attacks. Given all analyses revealed a significant main effect of drug, with nicotine decreasing all scores relative to saline-treated animals, planned comparisons were performed to isolate age-related differences within each treatment group. Data were analyzed utilizing a two way mixed-model design ANOVA with Age (3, PND 35, PND 45, PND 64) and Days as a repeated measure within each treatment (saline or nicotine).

Ambulation

Overall, time in ambulation varied as function of Age, Treatment and Day. In general, early adolescents showed greater time spent in ambulation relative to older animals as supported by a significant main effect of Age [$F(2, 37) = 5.24, p < 0.01$]. Time in ambulation changed across days as supported by a main effect of Days [$F(4, 148) = 10.01, p < 0.0001$].

As shown in Figure 2 (Panel A), the time spent in ambulation for the control animals was relatively stable over the five days within each age group. However, there was a significant age difference in time spent in ambulation in control animals [$F(2, 21) = 4.73, p < 0.05$]. For early adolescents in the control (saline-treated) group, on days one through five, the time in ambulation was higher than late adolescents ($p = 0.05$) and young adults ($p = 0.006$). For late adolescents, the time spent in ambulation increased slightly across days, but was not significantly

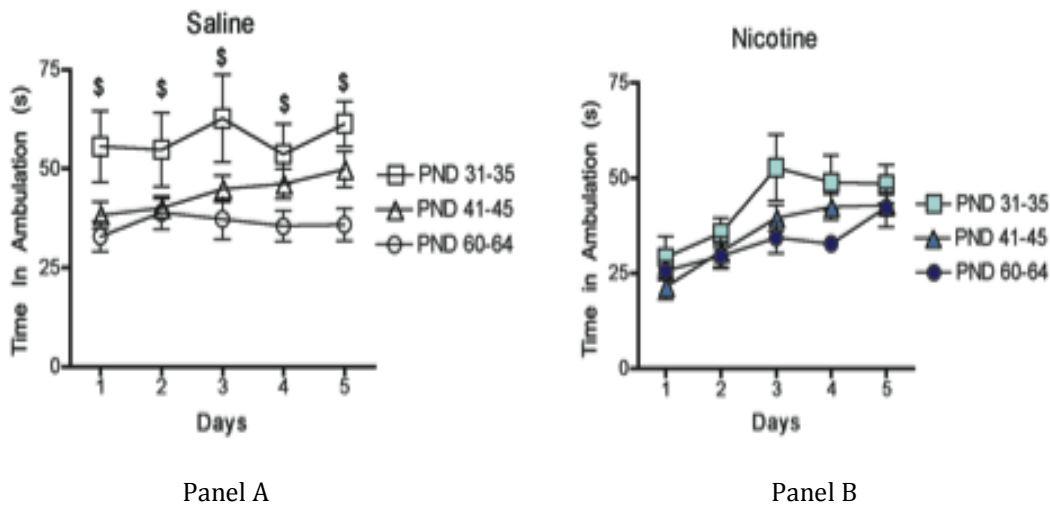


Figure 2 Shows the mean time (seconds) in ambulation +/- SEM of the experimental animals. Days one through five of testing are shown. The results for the control animals are shown on the Panel A, and those of the nicotine treated animals are shown on the Panel B. In Panel A and Panel B, square boxes represent early adolescents, triangles indicate late adolescent results, and circles represent the young adult animals. White shading in the boxes represents control (saline-treated) animals. In Panel B, light blue indicates early adolescents, teal, late adolescents, and dark blue, young adults. In Panel A, the \$ symbol indicates that, for the control animals, a main effect of age was observed, where the early adolescents spent more time in ambulation than the late adolescent and young adult animals

different from young adults. Young adults experienced the smallest change in time spent in ambulation but an overall increase was observed.

The changes in ambulation of the experimental (nicotine-treated) animals are shown in Panel B. In the animals that were administered nicotine, an initial depression in ambulation was observed, followed by an increase in ambulation [$F(4, 64) = 9.14, p < 0.0001$]. There were no statistically significant differences in time spent in ambulation in nicotine-treated animals across the five days of testing. For all five days of testing, nicotine-treated animals spent less time in ambulation than their control counterparts for each of the three age groups as supported by a main effect of Treatment [$F(1, 37) = 7.33, p < 0.05$] and a two-way interaction of Dose by Days [$F(4, 148) = 3.54, p < 0.01$].

Latency to Supine

In general, the time it took to be pinned (latency to supine) varied as function of Age, Treatment and Day. In general, young adults took longer to be pinned in the supine position relative to younger animals as supported by a significant main effect of Age [$F(2, 36) = 7.51, p < 0.01$]. Latency to supine position changed across days as supported by a main effect of Days [$F(4, 144) = 19.78, p < 0.0001$]. This varied across days as supported by a significant two-way interaction of Age by Days [$F(8, 144) = 2.01, p < 0.05$].

The time of latency to supine position, or the amount of time that passed before the experimental animal was pinned by the intruder, decreased in control animals over the five days of testing [F (4, 80) = 7.54, $p < 0.0001$] as shown in Figure 3 (Panel A).

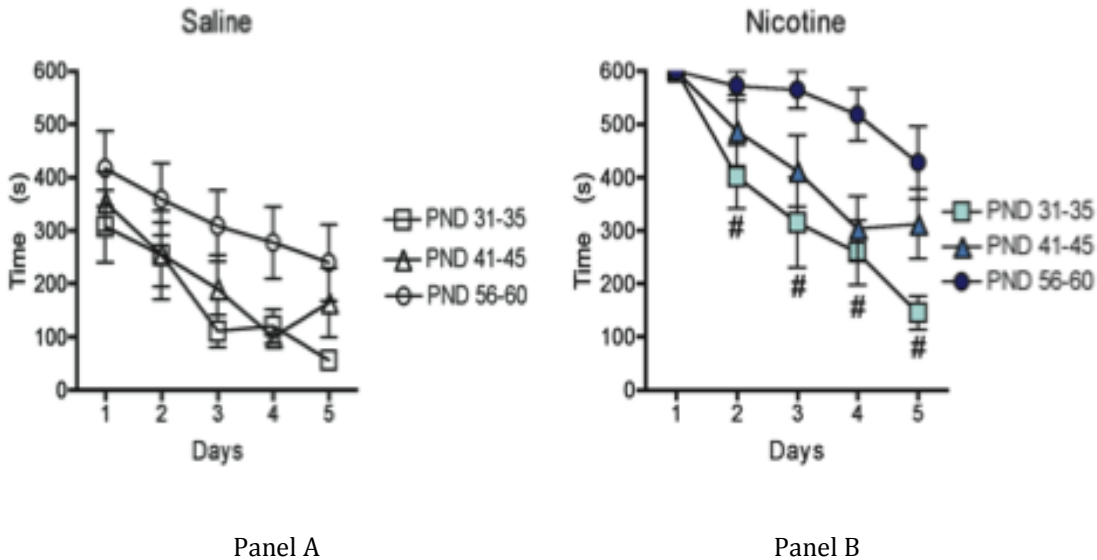


Figure 3 Shows the time of latency to supine position for the control and nicotine treated animals on days one through five of testing. The time is given in seconds. The results for the saline-treated animals are shown on the Panel A, and those of the nicotine treated animals are shown on the Panel B. In Panel A and Panel B, square boxes represent early adolescents, triangles, late adolescents, and circles represent the young adult animals. In Panel A, white shading in the boxes indicates results for control (saline-treated) animals. In Panel B, light blue indicates early adolescents, teal, late adolescents, and dark blue, young adults. In Panel B, the # indicates that a main effect of age was observed in the nicotine treated animals, where the early adolescents and late animals were pinned more quickly than the young adult animals.

In the nicotine-treated animals, (Panel B) a main effect of age was observed [F (2, 18) = 6.06, $p < 0.01$], where early adolescents ($p < 0.01$) and late adolescents ($p < 0.05$) were pinned more quickly than the young adult animals on all five days of testing. On day one of testing for each of the three age groups, the nicotine-treated animal was not pinned by the intruder animal. Over the five days test period, a decrease in the time of latency to supine position was observed, as was the case for the control animals, but for the nicotine-treated animals, the time of latency to supine was greater on each day of testing than it was for their control counterparts of the same

age group [$F(4, 72) = 15.18, p < 0.0001$]. In general, it took longer for nicotine-treated animals to be pinned relative to controls animals as supported by a main effect of Treatment [$F(1, 36) = 29.80, p < 0.0001$]. By comparing the time of latency to supine for the control and the nicotine groups, the age dependent effects of nicotine were observed. Independent of drug treatment, the early adolescents were pinned the most quickly, and the most time passed before the young adults were pinned.

Time in Supine

In general, the amount of time the animals were pinned (time in supine) varied as function of Age, Treatment and Day. In general, young adults were pinned for a shorter duration relative to young animals as supported by a significant main effect of Age [$F(2, 37) = 5.86, p < 0.01$]. Time in supine position changed across days as supported by a main effect of Days [$F(4, 148) = 13.93, p < 0.0001$]. This varied across days as supported by a significant two-way interaction of Age by Days [$F(8, 148) = 2.86, p < 0.01$].

For the time spent in supine position, or the amount of time that the experimental animal remained pinned on its back, in the control animals, a strong trend of a main effect of Age was observed [$F(2, 21) = 3.31, p = 0.056$], with early adolescents spending more time in the supine position than the young adult animals for all five days of treatment as shown in Figure 3 (Panel A). In control animals, time in supine increased across days [$F(4, 84) = 6.72, p < 0.0001$].

On each day of testing, the nicotine-treated animal in a given age group spent less time in the supine position than the control animals in the same age group, indicating a main effect of treatment [$F(1, 37) = 9.21, p < 0.01$]. On day one of testing, for nicotine treated animals in all three age groups, no time was spent in the supine position. In the nicotine treated animals, the same pattern of early adolescents spending the most time in the supine position and young adults spending the least time in the position was observed, showing that this pattern occurred independent of drug treatment. In the nicotine treated animals (Panel B), on days three, four, and five, the early adolescents spent more time in the supine position than the young adult animals,

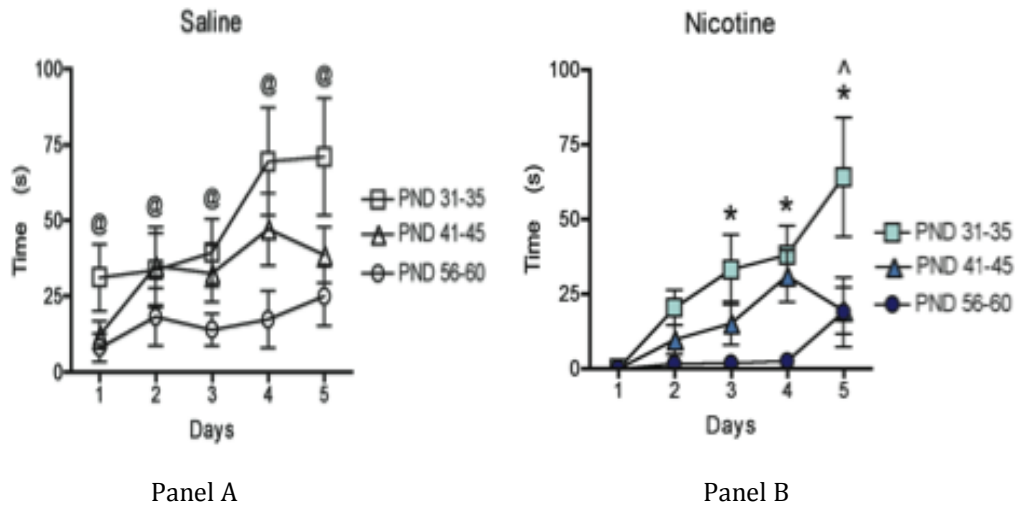


Figure 3 Shows the amount of time spent in the supine position, in seconds, over the five days of testing. In Panel A, the data for the control (saline-treated) animals is shown. Panel B contains the data for the experimental (nicotine-treated) animals. In both panels, square boxes represent early adolescents, triangles, late adolescents, and circles represent the young adult animals. In Panel A, white boxes indicate results for control (saline-treated) animals. In Panel B, light blue indicates early adolescents, teal, late adolescents, and dark blue, young adults. In Panel A, the @ symbol indicates that, for the control animals, a main effect of age was observed where early adolescents spent more time in the supine position than the young adult animals. In Panel B, the * on days three, four, and five of testing indicate that, for the nicotine treated animals, early adolescents spent more time in the supine position than the young adult animals. The ^ on day five indicates that the early adolescent animals spent more time in the supine position than the late adolescent animals.

indicating an age by days interaction [$F(,) = , p < .$]. On day one, the nicotine-treated animals in all three age groups spent no time in the supine position. Over the five days of the experiment,

the time in the supine position increased overall for the three nicotine-treated age groups, but the slope of this increase was greatest in the early adolescent animals and smallest in the young adult animals.

Pin Frequency

In general, the number of times the experimental animals were pinned (time in supine) varied as function of Age, Treatment and Day. In general, young adults were pinned for a shorter duration relative to young animals as supported by a significant main effect of Age [F (2, 37) = 16.16, p < 0.0001]. Time in supine position changed across days as supported by a main effect of Days [F (4, 148) = 216.79, p < 0.0001]. This varied across days as supported by a significant two-way interaction of Age by Days [F (8, 148) = 3.94, p < 0.001].

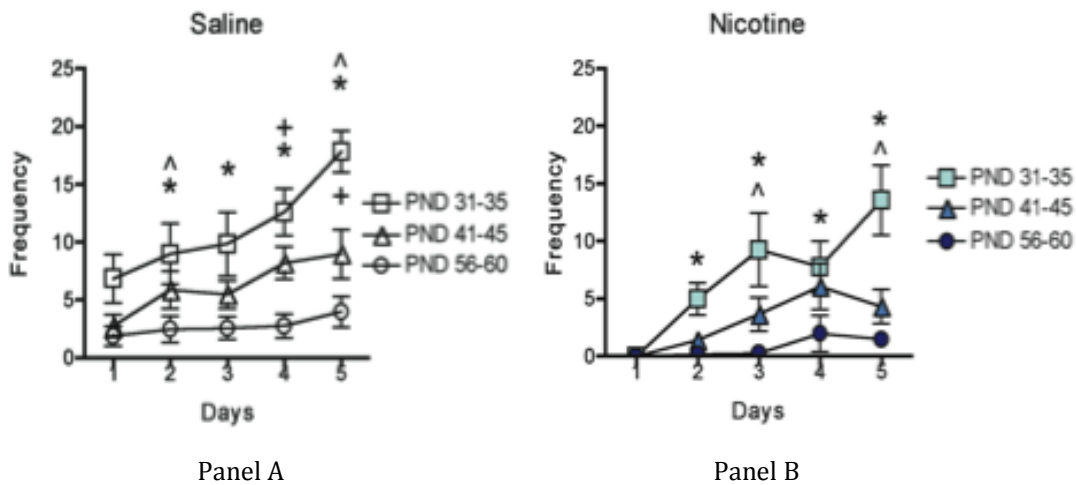


Figure 4 Shows the frequency of pins. This is the number of times that the intruder pinned the experimental animal. The results for control animals are shown in Panel A, and nicotine treated animals are in Panel B. In both panels, squares indicate early adolescent data, triangles represent late adolescent data, and circles show the young adult data. The white boxes in panel A give the data for the control animals. In panel B, the colored boxes indicated that data for the nicotine treated animals is shown, with early adolescents as light blue, late adolescents as teal, and young adults as dark blue. In Panels A and B, for days two through five of testing, the * indicates that, for both the control and experimental groups, early adolescents were pinned more frequently than the young adult animals. In Panel A, the ^ indicates that early adolescents were pinned more frequently than the late adolescent animals, and the + on days four and five indicates that on those days, the late adolescents were pinned more frequently than the young adults.

Frequency of pins is shown in Figure 4. On days two and five, in the control group, early adolescents were pinned more frequently than late adolescents, and on day four, late adolescents were pinned more frequently than young adults, indicating an age by days interaction [$F(4, 84) = 2.41, p < 0.05$] (Panel A). For early adolescent and late adolescent animals, the number of times they were pinned increased over the five day test period, and for the young adults, the frequency of pins only increased slightly from day four to five, indicating a main effect of Age [$F(21, 21) = 11.56, p < 0.0001$]. Frequency of pins changed across Days [$F(4, 84) = 10.21, p < 0.0001$] in control animals.;

For the nicotine-treated animals (Panel B), early adolescents were pinned more than late adolescents and young adults as supported by a main effect of Age [$F(2, 18) = 8.87, p < 0.01$]. On days three and five, an Age by Days interaction [$F(8, 72) = 2.48, p < 0.05$] was observed where early adolescents treated with nicotine were pinned more frequently than late adolescents treated with nicotine. On test day one, in all age groups, no animal administered nicotine was pinned. Over the five day test period, the frequency of pins increased, and the slope of the increase was greatest for the early adolescent animals and smallest for the late adolescent animals [$F(4, 72) = 9.01, p < 0.0001$]. A overall main effect of Drug was observed [$F(1, 37) = 15.70, p < 0.001$], where in each age group, the frequency of pins was fewer than those of the control counterpart in the corresponding age group for all five days.

Attacks

Overall, the number of attacks made to the intruder varied as function of Age, Treatment and Day. In general, early adolescents and late adolescents showed greater number of attacks to the intruder relative to young adults as supported by a significant main effect of Age [$F(2, 36) =$

6.75, $p < 0.0001$]. Number of attacks changed across days as supported by a main effect of Days [$F(4, 144) = 25.27, p < 0.0001$].

For control animals (Panel A), a main effect of age was observed [$F(2, 21) = 9.44, p < 0.001$] in the frequency of attacks made by the experimental animal against the intruder animal, where early adolescents and late adolescents attacked intruders more frequently than the young adults on all five days of testing. Frequency of attacks changed across the five test days as supported by a significant main effect of Days [$F(4, 84) = 12.76, p < 0.0001$]. The data are shown in Figure 5. Over the five days of testing, the frequency of attacks increased for control animals in each age group, with the greatest increase occurring in early and late adolescent animals, and the smallest increase occurring in the young adult animals.

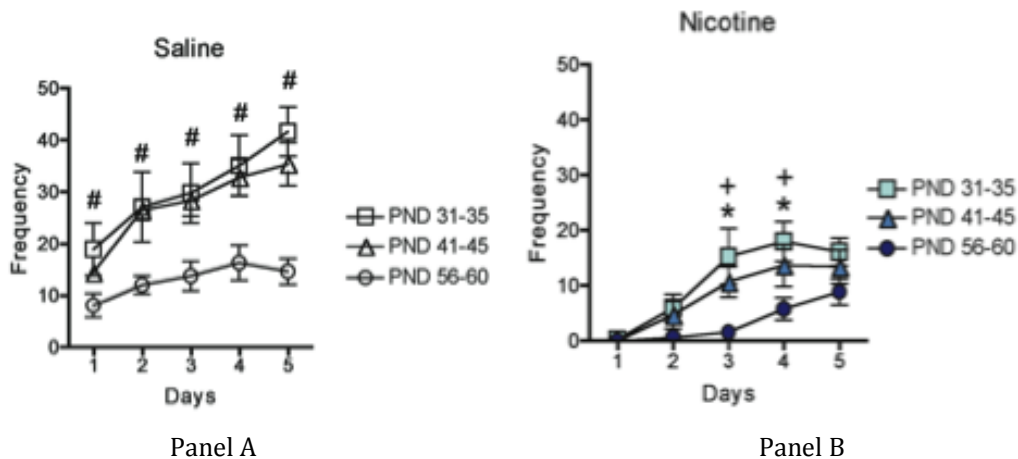


Figure 5 Shows the number of times that the experimental animal attacked the intruder animal for each of the five days of testing. The results for the control animals are shown on the Panel A, and those of the experimental animals are shown on the Panel B. In Panel A and Panel B, square boxes represent early adolescents, triangles, late adolescents, and circles represent the young adult animals. In Panel A, white shading in the boxes indicates results for control (saline-treated) animals. In Panel B, light blue indicates early adolescents, teal indicates late adolescents, and dark blue indicates young adults. In Panel A, the # indicates that, for the control animals, a main effect of age was observed where the young adults attacked the intruder less frequently than the early adolescent or late adolescent animals. For the experimental animals, in Panel B, on days three and four of testing, the + indicates that late adolescents attacked the intruder more frequently than the young adult animals and the * indicates that the early adolescent animals attacked the intruder more frequently than the young adult animals.

In the nicotine-treated animals (Panel B), on days two and three of testing, early adolescents and late adolescents were pinned more frequently than the young adult animals, indicating a main effect of Age [$F(2, 15) = 6.75, p < 0.01$], a significant main effect of Days [$F(4, 60) = 16.22, p < 0.0001$] and a significant two-way interaction of Age by Days [$F(8, 60) = 2.15, p < 0.05$]. In the overall analysis, a main effect of Treatment was also observed [$F(1, 36) = 41.78, p < 0.0001$], such that on each day of testing, the number of attacks performed by the nicotine-treated animals was lower than the number of attacks made by the control animals in the same age group.

Overview of Results

Overall, all behaviors increased across days for early adolescents, but the slope of this increase was blunted by nicotine treatment. There was an increase in responsivity to repeated social stress in control animals, but this was blunted by treatment with nicotine. The increase was also age dependent. Younger animals were more responsive to repeated social stress than older animals, whether they received nicotine or saline.

Discussion

Analysis of the collected data indicated that nicotine treatment depressed or decreased all behaviors under analysis. The amount of time in ambulation was less for the animals that were administered nicotine than their saline counterparts. More time passed before the nicotine treated animals were pinned than for the control animals to be pinned, the nicotine treated animals were pinned less frequently and stayed pinned for a shorter amount of time than the control animals in the corresponding age group. Additionally, the animals that were treated with nicotine attacked the intruder less frequently than the control animals. These results all indicate that nicotine

decreased aggressive behavior and general activity. It is likely that the nicotine-treated animals were pinned less frequently, took longer to be pinned, and remained pinned for a shorter amount of time because they spent less time in ambulation, in other words, their general activity was depressed, and because they attacked the intruder animals less often than the control animals, they were pinned less frequently and for a shorter amount of time.

The results of previous experiments indicate that in both humans and male rats, those administered nicotine and then exposed to a social stressor respond in a more aggressive way than those that are not administered nicotine (File et al., 2001; Clarke and Kumar, 1983a). Similar findings were expected in this study, however opposite effects were observed. Instead, nicotine treatment decreased activity and depressed aggressive behavior. The dose of nicotine that was administered in the present research experiment, 0.6 mg/kg, was relatively high and depressed activity in all animals, regardless of age. This dose in rats contains approximately the same amount of nicotine as would be consumed by a moderate to heavy smoker that smokes about a pack of cigarettes a day (Pentel et al., 2000). Rather than beginning with a small dose and increasing the dose each day, the initial dose of nicotine administered was very high. This may have caused the rats to experience the depressant effects of nicotine, rather than stimulant effects (Clarke and Kumar, 1983a; Clarke and Kumar, 1983b). For this reason, future experiments should investigate dose dependent alterations in reactivity to social stress. This would allow the stimulatory and sedative effects of nicotine to be studied and to determine at what dose the effect changes.

The early adolescent animals in the nicotine-treated group were pinned more frequently and more quickly than the late adolescents and young adults in the nicotine group, possibly because they attacked the intruder animal more frequently than the late adolescents or young

adults. In both control and nicotine groups, early adolescents were the most active and responded the most aggressively to the social stressors and the young adults were the least aggressive and active. In the nicotine-treated groups, each of the five factors was depressed, but the relationship between the three age groups remained. This indicates that the reactivity to social stressors is age dependent and maintained in a drug dependent fashion.

Over the five-day test period, all behaviors increased for the early adolescents, but the slope of this increase was blunted by treatment with nicotine. In future work, the dose of nicotine administered should be lower to enable the analysis of the early stimulatory effects of nicotine on age-dependent changes in responsiveness. Also, testing should be performed into adulthood. The five-day test period was relatively short. If the testing occurred for a longer amount of time, stimulant effects may have been observed, as the anticipated sensitizing effects of nicotine began to exhibit (DiFranza & Wellman, 2007; Clarke & Kumar, 1983a; Clarke and Kumar, 1983 b).

In future studies, the responsivity to non-aggressive stressors should also be examined. Human adolescents face a variety of stressors, both aggressive and non-aggressive. If the same dose of nicotine were used, studying the response to non-aggressive social stressors in rats would allow for comparison of the responses because adolescent and young adult animals may respond differently to non-aggressive stressors (Daniels et al., 2000). The findings could then be extrapolated and applied to humans. The responses must be understood in order for successful smoking cessation programs to be developed. Two non-aggressive social stressors that could be studied in rats are overcrowding, in which multiple rats live in one cage, and unstable housing, in which the experimental animal is placed in a different cage every day which inhibits the development of a hierarchy within the animals in a given social environment. These factors are both models for the human stressors that many adolescents face, whether they live in foster

homes, in separate homes with divorced parents, or with multiple people in a very small space (Daniels et al., 2000). These are some of the stressors that may cause adolescents to begin using tobacco products, and studying the response of rats in these conditions to treatment with nicotine would again allow for the development of better smoking cessation programs.

In recent years, many nicotine studies have aimed to determine whether or not sensitization occurs in adult male and female rats. The degree of these changes has been measured using quantitative changes in locomotor activity and the most significant developmental research has only investigated whether or not the sensitization that is experienced continues into adulthood. Results of human and animal nicotine research were used to construct an experiment in which the relationship between nicotine administration and changes in activity level and aggression in adolescent male rats could be studied. It is known that nicotine enhances aggressive behavior in stressed human adolescent males, while inducing a calming effect in stressed females (File et al., 2001). The goal for the present study was not only to observe changes in activity and aggression in male rats, but also to determine if any variation in responsivity with age occurs by testing early adolescent, late adolescent, and young adult males. The decreases in aggressive responses and activity were not expected, but testing three different age groups of rats did allow for detection of the age-dependent effects of nicotine treatment, with early adolescents being the most active and aggressive and young adults being the least active and aggressive.

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