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INDIVIDUAL DIFFERENCES IN ORAL NICOTINE INTAKE IN RATS

Tanseli Nesil^{1,2}, Lutfiye Kanit^{1,3}, Allan C Collins⁴, and Sakire Pogun^{1,*}

¹ Ege University, Center for Brain Research

² Ege University, Institute of Sciences, Biotechnology Dept

³ School of Medicine, Physiology Dept., Bornova, Izmir, Turkey

⁴ Institute for Behavioral Genetics, University of Colorado, Boulder, Colorado, USA

Abstract

To study individual differences in nicotine preference and intake, male and female rats were given free access to a choice of oral nicotine (10 or 20 mg/L) or water for 24 hours/day for periods of at least six weeks, starting at adolescence or adulthood. A total of 341 rats, were used in four different experiments; weight, nicotine intake and total liquid consumption were recorded weekly. Results show that rats can discriminate nicotine from water, can regulate their intake, and that there are readily detected individual differences in nicotine preference. Ward analyses indicated that the animals could be divided into minimum, median and maximum preferring subgroups in all experiments. The effect of saccharine on nicotine intake was also evaluated; although the addition of saccharine increased total intake, rats drank unsweetened nicotine solutions and those with higher preferences for nicotine, preferred nicotine over water with or without saccharine added. Nicotine reduced weight gain and the effect was more pronounced in females than males. The average nicotine consumption of adolescent rats was higher than adults and nicotine exposure during adolescence reduced nicotine intake in adult rats. About half of the rats which had access to nicotine as adolescents and also as adults had a persistent pattern of consumption; the behavior was very stable in the female minimum preferring groups and a much higher ratio of rats sustained their adolescent behavior as adults. The change in preference was more pronounced when there was an interval between adolescent and adult exposure; female rats showed a more stable behavior than males suggesting a greater role for environmental influences on males. In conclusion, marked individual differences were observed in oral nicotine intake as measured in a continuous access 2-bottle choice test. Age and sex of the subjects and previous exposure to nicotine are significant factors which affect preference in rats.

Keywords

nicotine; individual differences; sex; age; adolescent; oral self-administration; rat

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*Corresponding author: Prof. Dr. Sakire Pogun, Ege University, Center for Brain Research, Bornova, 35100, Izmir, Turkey, Telephone: +90 232 3882868, Telefax: +90 232 3746597, sakire.pogun@ege.edu.tr.

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1. INTRODUCTION

Individual differences in tobacco addiction have been identified in many studies (Perkins, 1995; Pomerleau, 1995; Pomerleau et al., 1993; Shiffman, 1989; Shiffman and Paton, 1999). Genetic factors (reviewed in (Bierut et al., 2007; Portugal and Gould, 2008)), including sex of the subject (Perkins and Scott, 2008; Pogun and Yazarbas, 2009), contribute to these individual differences. It is clear that nicotine plays a major role in regulating the reinforcing effects of tobacco given that nicotine-containing cigarettes produce more intense positive subjective effects than de-nicotinized cigarettes (Donny et al., 2007; Gross et al., 1997; Robinson et al., 2000) and alleviate more withdrawal symptoms (Buchhalter et al., 2005). These findings make sense since it is well established that nicotine has direct effects on brain regions that play a major role in modulating reinforcement, such as the ventral tegmental-nucleus accumbens (Mansvelder et al., 2007; Wooltorton et al., 2003). However, it has become increasingly apparent that the reinforcing effects of tobacco are modulated by other factors such as taste (Dehkordi et al., 2010).

Animal models have been used extensively in studies designed to broaden our understanding of those mechanisms that play important roles in modulating nicotine addiction. Intravenous nicotine self-administration has been studied extensively to identify mechanisms that influence the reinforcing effects of nicotine. Four decades ago, Clark (Clark, 1969) showed that rats self administer nicotine by oral and i.v. routes and suggested that nicotine may be the “appealing” constituent of tobacco. Corrigan and Coen (Corrigan and Coen, 1989) confirmed this suggestion by demonstrating that rats (male, Long Evans) will self-administer nicotine intravenously using a 1-hour limited access paradigm. Although there are studies which employed continuous nicotine exposure for as long as five weeks in adult rats using i.v. self administration (Cox et al., 1984; Valentine et al., 1997), to the best of our knowledge no study has evaluated nicotine intake in the same animal starting from adolescence and continuing through adulthood for as long as 23 weeks. The present study was undertaken to develop an animal model which closely mimics the human smoking condition, especially regarding free access to and longer duration of nicotine exposure compared to most of the reported findings in literature.

Supplying nicotine in drinking water has been used to study chronic nicotine exposure in rodents (Rowell et al., 1983) and has become a widely accepted method of self administration. When rodents are given a choice of water or a nicotine solution, consumption shows individual variability, including sex and age differences (Abreu-Villaca et al., 2006; Adriani et al., 2002a, b; Biondolillo and Pearce, 2007; Dadmarz and Vogel, 2003; Flynn et al., 1989; Glatt et al., 2009; Isiegas et al., 2009; Kameda et al., 2000; Klein et al., 2004; Lee et al., 2004; Li et al., 2007; Maehler et al., 2000; Marshall et al., 2003; Pawlak and Schwarting, 2002; Robinson et al., 1996; Todte et al., 2001; Vieyra-Reyes et al., 2008). When nicotine is administered orally, the blood levels do not rise as quickly as intravenous application, subsequently the effects are relatively more subtle. Nicotine in the drinking water that is not absorbed immediately in the mouth is metabolized in the liver during the first-pass. Therefore, compared to i.v. self administration in rodents or inhalation in humans, the rate of entry to the brain is slower and the quantities are lower and more variable (Matta et al., 2007). On the other hand, free access and extended exposure periods are major advantages. Studies on the reinforcing effects of nicotine using the oral route of administration suggest that blocking nicotinic and dopaminergic (D4) receptors may reduce the reinforcing effect of nicotine (Kameda et al., 2000) and that the nAChR subunit genes mediate in sensitivity to nicotine (Li et al., 2007; Portugal and Gould, 2008; Robinson et al., 1996). Oral nicotine has also been considered for smoking cessation in human smokers. An oral nicotine solution, when mixed with a beverage was well tolerated and served as nicotine replacement (Westman et al., 2001).

Oral nicotine self administration studies done with the mouse clearly show that genetic factors influence this behavior. These genetic effects are seen when inbred mouse strains are tested using a 2-bottle choice procedure (Li et al., 2007; Portugal and Gould, 2008; Robinson et al., 1996) and when F2 hybrids derived from inbred strains are tested (Butt et al., 2005; Li et al., 2007). Many studies (see, for examples: (Biondolillo and Pearce, 2007; Flynn et al., 1989; Pawlak and Schwarting, 2002; Vieyra-Reyes et al., 2008)) have used the rat to study oral nicotine self-administration, but most of these studies used a single strain. Nonetheless, comparisons of the results obtained in these studies suggest that inbred and outbred rat strains differ in oral nicotine intake. The mouse and rat studies published, to date, have limitations, however. For example, nearly all of the rodent oral self-administration studies used young adult male animals, and most of the studies used 1–2 week evaluation periods. The studies described in this paper were designed to address several important questions:

- Does oral intake change with age?
- Does exposure to nicotine in the drinking water as an adolescent affect oral intake of nicotine as an adult?
- Does sex influence oral nicotine intake?
- Is oral self-administration a stable behavior?
- Does the addition of saccharine alter nicotine intake?

We addressed these questions in four experiments. In the first experiment, male and female Spague-Dawley rats were provided with a choice of oral nicotine or water for 24 hours/day for 23 weeks, starting at 5 weeks of age (adolescence). In the second experiment, adolescent rats of both sexes were tested for two 6-week periods, one starting at 5 weeks of age (adolescence) and another starting 10 weeks after the adolescent testing had ended. Adult rats were tested for 6 weeks (starting either at 10 or 17 weeks of age) in the third experiment. Our study differs from previous studies because we have used much longer exposure periods and have studied adolescent and adult consumption patterns in the same rats. The design of the experiments enabled us to compare the effect of age at onset, previous exposure and continuous versus intermittent exposure to nicotine. Animal weight was monitored weekly to determine whether nicotine exposure affected weight gain. We used outbred male and female Sprague-Dawley rats to evaluate potential sex differences on these measures as detected by individual differences.

Our major goal in conducting the series of experiments presented here was to establish a method of nicotine self administration that closely mimics the human smoking conditions. Our first hypothesis was that there will be very significant individual differences in nicotine intake, including sex differences. We also hypothesized that adolescent rats and females will have greater intake than adults. Previous reports have suggested that adolescent rats exposed to nicotine for four weeks reduce intake (Levin et al., 2007; Levin et al., 2003); however, it was hard to make predictions on the stability of intake for longer periods, because reported longitudinal data on rodents is very limited. These are important and timely questions considering the high rates of initiation of smoking among adolescents despite the decline in smoking among adults. Since our experiments are designed to mimic human smoking, our results may have translational impact.

2. MATERIAL AND METHODS

2.1. Laboratory Animals

Male and female adult Sprague Dawley rats, obtained from Ege University Laboratory Animal Breeding Facility were bred in our laboratories, i.e. pregnant rats were observed and

the age of the animals in the experiments was determined by their birth date. All the rats were under identical conditions and were handled similarly during all stages of development. The onset of puberty is accepted to be at 40–42 days of age in rats and adolescence continues until approximately 60 days of age. Periadolescence is described as 10 days before puberty and rats are designated as adults after 60 days of age (Izenwasser, 2005; Spear and Brake, 1983). Based on this description, we started nicotine treatment in adolescent rats at 5 weeks of age (~35 days) and in adult rats at either 10 weeks (~70 days, young adult) or 17 weeks (~120 days) of age.

This strain was selected for study because it is an outbred strain. After weaning (~4 weeks), rats were housed singly in standard laboratory conditions (20–22 °C, 12–12 hours light-dark cycle), fed ad libitum (standard rat food pellets) and received their drinking solutions from two bottles at all times.

The animals were treated under the prescriptions for animal care and experimentation of the pertinent European Communities Council Directive (86/609/EEC), and all the procedures were approved by the Institutional Animal Ethics Committee of Ege University.

The data presented in the current study were obtained from five different sets of animals in four different experiments (Figure 1).

1. Adolescent, continuous: Rats were exposed to oral nicotine/water (n=66; 36 female) or water/water (controls, n=22; 10 female) starting at 5 weeks of age (adolescents); this exposure continued (23 weeks) without interruption through adulthood.
2. Adolescent, interrupted: Rats were exposed to oral nicotine/water (n=67; 43 female) or water/water (controls, n=21; 10 female) starting at five weeks age for a total duration of six weeks. Then there was an interval of nine weeks where all of the animals were provided with water/water. After the nine week interval, rats were again provided with a nicotine/water (water/water for controls) choice for six weeks as adults where intake was measured again.
3. Adult, 10 weeks: Rats were exposed to oral nicotine/water (n=27; 12 female) or water/water (controls, n=16; 8 female) as young adults (10 weeks of age) for six weeks. Adult, 17 weeks: Rats were exposed to oral nicotine/water (n=67; 40 female) or water/water (controls, n=16; 8 female) as adults (17 months of age) for six weeks.
4. Adult (17 weeks), saccharine effect: Effect of saccharine on nicotine intake was evaluated by initially exposing the rats to nicotine/water solutions without saccharine (1&2 weeks 10mg/L, 3&4 weeks 20 mg/L nicotine) then adding saccharine to both solutions (weeks 5&6, 20 mg/L nicotine) and finally removing saccharine (7&8 weeks, 20 mg/L nicotine) (n= 39, 20 female)

Collected data were analyzed to determine whether:

1. rats exposed to nicotine under identical conditions have different preferences for nicotine
2. age (adolescent versus adult) of first exposure affects oral nicotine consumption.
3. exposure patterns (continuous versus intermittent) affect nicotine intake.
4. previous exposure to nicotine (during adolescence) affects adult nicotine consumption (i.e. comparing nicotine consumption of adult rats with or without previous nicotine exposure)

5. rats can discriminate nicotine from water and regulate their intake accordingly.
6. nicotine intake had significant effects on weight gain.
7. saccharine has a significant impact on nicotine intake

2.2. Drugs

Rats received nicotine [Sigma, (–) nicotine hydrogen tartrate] and water in two bottles. The concentration of nicotine was 10 mg/L during the initial two weeks of nicotine exposure. The nicotine concentration in the drinking water was raised to 20 mg/L on day 15 and was kept constant for the rest of the experiment. Nicotine concentration used was comparable to published reports using similar protocols in rats (Adriani et al., 2002a; Smith and Roberts, 1995). Control rats received only water from both bottles. Saccharine sodium (20 mg/L) was added to both the nicotine and water bottles in an attempt to make the nicotine less aversive in most of the experiments (Sparks and Pauly, 1999).

The bottles were placed on either side of the food pellet compartment, so that the distance of each bottle to the food and to the side of the cage was the same. This arrangement prevented the possible bias which may arise from drinking more liquid from the bottle closer to food. After weaning, there were always two bottles on the cages to get the rats accustomed to two bottles. In preliminary experiments, we found that identical data were obtained when the bottles were rotated from side-to-side and when the bottles were left in the same position; therefore the position of the bottle containing nicotine was not changed in the experiments. However, in all experiments bottle position was counterbalanced between animals although no significant differences were observed. Control rats drank equal amount of liquid from both bottles (not different from 50%). Although rats drank more liquid as they grew (from adolescence to adulthood) and males drank larger volumes than females, the total amount of liquid consumed was similar between the groups (107 ± 5 and 129 ± 6 ml, adolescent females and males; 147 ± 9 and 166 ± 8 , adult females and males). The effect of saccharine on liquid intake is provided in the results section. Possible leakage from or clogging of the tip of the bottles were controlled using three different measures: checking bedding, comparing the total liquid the rats drank the previous week and the average amount of liquid rats in the same group drank. If a problem was identified, the measurements from the particular animal for that week were not recorded. However, loss of data was minimal and constituted about 1–2 % of total measurements. There was always an empty cage with two sham bottles in the same position as the rat cages in the animal facility, matched to drinking conditions to determine possible loss by evaporation and handling. The loss never exceeded 0.4 ml/week and did not show variability over weeks. The average loss (0.24 ± 0.06 ml) was negligible; therefore no correction was employed in the data presented.

2.3. Data collection

Animals were weighed, and the volumes of the solutions they consumed (nicotine and water) from both bottles were measured weekly. Total liquid taken and nicotine consumption (mg/kg/week) were calculated from these weekly data. Results showed that rats could always be divided into three different groups (Ward method, $p < 0.01$) based on their average nicotine consumption over six week periods. Subsequently this grouping was used in the studies presented: For data analyses, male and female rats were separately grouped as maximum, median and minimum nicotine preferring rats according to their average weekly nicotine consumption over six week periods of oral nicotine self administration.

2.4. Statistical Analysis

The Ward cluster method (Ward, 1963) was used to group the rats according to their differing preferences for nicotine (maximum, median and minimum).

Comparisons were performed using the Kruskal-Wallis test, and the significance was set at $p < 0.05$.

Repeated measures ANOVA was performed with percent liquid from the nicotine side or the amount of nicotine consumed as the dependent variable, and sex (female, male) age (adolescent, adult) and Ward groups (minimum, median, maximum, control) as the between subjects factors. In some analyses, weeks was also included as the within-subjects factor. LSD and Bonferroni tests were used for post hoc analyses as required. Further detail on statistical analyses is provided in the “Results” section.

3. RESULTS

3.1. Time course of nicotine consumption over 23 weeks

Figure 2 shows the time course of nicotine consumption in rats which had free access to a nicotine choice for 23 weeks, starting at five weeks of age (continuous regimen). As depicted in Figure 2, nicotine consumption declines significantly with age. Repeated measures ANOVA with nicotine consumption as the dependent variable, weeks (age 5–28 weeks) as the within-subjects and sex (female, male) as the between-subjects factors revealed a significant effect of weeks of exposure on nicotine consumption [$F(23, 138) = 21.367, p < 0.001$].

No correlation was observed between nicotine consumption and total liquid intake.

3.2 Comparison of nicotine consumption in rats with different age of onset of nicotine exposure

To evaluate the effect of age of initial exposure to nicotine on nicotine consumption, rats that were given a free choice of nicotine/water starting at adolescence (5 weeks of age) were compared with those who were exposed to nicotine as adults (10 or 17 weeks of age). No sex differences were observed and therefore males and females were pooled; average nicotine consumption (mg/kg/week) over six weeks was 5.80 ± 0.29 , 3.85 ± 0.35 , and 4.66 ± 0.35 for adolescents, young adults and adults, respectively. Repeated measures ANOVA with weeks (1–6) as the within-subjects and starting age (5, 10, 17 weeks) as the between subjects factors revealed significant effects of weeks [$F(5, 219) = 3.555, p < 0.005$] and starting age [$F(2, 219) = 4.643, p < 0.01$], as well as an interaction [$F(10, 219) = 2.216, p < 0.05$]. A one way ANOVA with average nicotine consumption as the dependent variable and starting age (5, 10, 17 weeks) as the grouping factor revealed a significant difference between the groups [$F(2, 158) = 6.712, p < 0.05$]; post hoc Bonferroni tests showed that rats exposed to nicotine at adolescence consumed more nicotine than those exposed as young adults ($p < 0.005$) and adults ($p < 0.05$). Figure 3 shows the pattern of nicotine consumption over six weeks. One way ANOVA for each week, with age as the grouping variable and nicotine consumption as the dependent variable, depicted differences between the groups on weeks 1 [$F(2, 224) = 5.484, p < 0.005$], 2 [$F(2, 224) = 3.386, p < 0.05$], 4 [$F(2, 222) = 3.300, p < 0.05$], and 5 [$F(2, 224) = 3.092, p < 0.05$].

3.3 Comparison of nicotine consumption in adult rats that were or were not exposed to nicotine previously

A univariate ANOVA was performed with average weekly nicotine consumption over six weeks as the dependent variable, groups (continuous, interrupted, adult) and sex (female,

male) as the between subjects factors. A significant main effect of groups was observed [$F(2,199)=44.467$, $p<0.001$]. Sex did not emerge as a main effect and no interactions were observed. As depicted in Figure 4, post-hoc Bonferroni test showed that each group was significantly different from the other two groups. These results indicate that nicotine consumption in adult rats is highly dependent on previous nicotine exposure and while rats with continuous exposure consume the least, rats exposed to nicotine as adults consume the most nicotine.

3.4. Individual differences in nicotine preference

A hierarchical cluster analysis was used to partition the rats, based on average weekly nicotine consumption, into homogeneous subgroups. Ward's method (Ward, 1963) is employed to determine the optimum number of clusters in the data set. Then individual rats are partitioned to a particular cluster based on their nicotine consumption, consequently variance within a cluster is low. The results of the hierarchical cluster analysis using Ward's method described above produced three subgroups (maximum, median, minimum) in all of the experiments (Table 1). Subsequently, the difference between the three subgroups for each experiment was always significant ($p<0.001$ in all cases).

One way ANOVAs for different experimental groups/regimens within the same Ward subgroup, same age and sex all revealed significant differences at the $p<0.001$ level. Adult females, minimum groups: $F(3,48)=19.419$, median groups: $F(3,41)=31.024$, maximum groups: $F(3,51)=22.112$; males, minimum groups: $F(3,29)=8.090$, median groups: $F(3,29)=170.175$, maximum groups: $F(3,26)=66.417$. These results show that data derived from rats exposed to nicotine using different regimens (interrupted or continuous starting at adolescence, young adulthood and adulthood) are all divided into three subgroups (minimum, median, maximum) as adults and that the nicotine consumption within the same preference subgroup are significantly different from each other. The results of post-hoc Bonferroni tests are shown in Table 1. Please note that in longitudinal experiments the adolescent rats evaluated also as adults, are not always in the same Ward group.

Since the adolescent rats in the two different regimens (interrupted and continuous) were treated similarly during the initial six weeks of nicotine exposure, there were no differences between these two groups; the effect of different regimens emerged only in adult rats.

The distribution and number of rats exposed to different treatment regimens during adolescence and adulthood is shown in Figure 5. The graphs depict considerable variation in the patterns of nicotine consumption in rats.

As shown above, in all of the experiments, rats could be separated into 3 groups by Ward analyses. Furthermore, while some of the rats showed a steady pattern in nicotine consumption (mainly minimum and maximum nicotine preferring rats), others had occasional deviations from their average pattern. The rats with sporadic variation almost always fell in the "median" groups. The table below (Table 2) is intended to give the reader an idea about the raw data and shows individual data from 16 rats over six weeks starting from the initial exposure to oral nicotine self administration.

To study the persistence of nicotine preference as rats develop, nicotine consumption patterns of rats which were exposed to nicotine during both adolescence and adulthood (continuous and interrupted regimens) were analyzed during the initial (adolescence) and final six weeks (adulthood) of nicotine exposure. The trends in nicotine consumption are shown in Table 3. Comparing the Ward groups (minimum, median, maximum) during adolescence and adulthood revealed trends: some animals increased or decreased their nicotine preference as they grew older while there were some rats whose preference did not

change with age. Fisher exact analyses revealed significant differences between the trends of the groups in the interrupted ($p < 0.01$), but not in the continuous regimen. Chi square analyses depicted a significant change between males and females for the interrupted group ($p < 0.01$) because from adolescence to adulthood the number of male rats which increased their nicotine preference (not actual nicotine consumption, but their place within the same age group according to preference) was significantly higher than females and the number of female rats which decreased their nicotine preference was significantly higher than males; furthermore, almost half of the female rats stayed at the same level while a much smaller portion of male rats stayed at the same level of preference. Although there was a similar trend in the continuous regimen group, the difference did not reach significant levels ($p < 0.10$). These findings indicate that the change in pattern of nicotine consumption is more pronounced when there is an interval between adolescent and adult exposure and while females reduce their preference, males increase it. Furthermore, in the continuous exposure regimen, 57% of females and 48% of males, and in the interrupted regimen 42% of females and only 17% of males persist on their pattern of nicotine preference from adolescence through adulthood.

Figure 6 shows the persistence or change in the pattern of nicotine consumption as rats mature from adolescence to adulthood. As depicted in the figure, minimum nicotine preferring female rats have the most consistent pattern from adolescence to adulthood.

3.5. Testing if rats can discriminate nicotine and regulate their liquid intake from the bottle on the nicotine side accordingly

The percent of the total liquid that rats (continuous exposure group) drank from the nicotine side (for controls, water from the same side) was compared in minimum, median, and maximum nicotine preferring rats that were exposed to nicotine/water (grouped according to Ward analyses) and control animals that received water/water, starting at adolescence. Data were analyzed for adolescence (5–11 weeks) and adulthood (22–28 weeks). The percent liquid measurements were taken as the average of two week periods since nicotine concentration was 10 mg/L for the initial two weeks and 20 mg/L for the remaining period. Repeated measures ANOVA was performed with percent liquid rats drank from the nicotine side as the dependent variable, weeks (1–2, 3–4, 5–6) as the within-subjects factor, and sex (female, male), age (adolescent, adult) and Ward groups (minimum, median, maximum, control) as the between subjects factors. Significant effects were observed for all of the factors: weeks [$F(2,475) = 9.036$, $p < 0.001$], sex [$F(1,475) = 4.609$, $p < 0.05$], age [$F(1,475) = 92.607$, $p < 0.001$] and Ward group [$F(1,475) = 120.629$, $p < 0.001$]. Briefly, the percent liquid rats drank from the nicotine side decreased through weeks, females drank less than males, adolescent rats drank more than adults and drinking varied according to the Ward group, such that Minimum < Median < Maximum. There was a significant weeks $\times$ age interaction [$F(1,475) = 15.933$, $p < 0.001$] because the change in percent liquid through weeks was observed only in adolescents but not in adults. Considering that the nicotine concentration was the same during the adult period, this finding reflects the regulation of nicotine uptake when nicotine concentration is altered. One way ANOVAs, followed by post-hoc Bonferroni tests, were performed for different Ward groups, separately for each age and sex group to depict the differences between the three two-week periods. There were significant differences between the weeks in the adolescent female, minimum [$F(2,76) = 14.873$, $p < 0.001$] and median [$F(2,76) = 6.100$, $p < 0.005$]; adolescent male minimum [$F(2,99) = 9.003$, $p < 0.001$] and median [$F(2,46) = 5.676$, $p < 0.01$] groups. There was no difference between the weeks in the other groups. None of the control groups was different from 50%, indicating that one bottle was not preferred over the other when both contained water. Since within the same group no significant differences between the percent liquid rats drank from the nicotine side was observed in adults, Figure 5 shows only the percent liquid

taken during the adolescent period. Similar results were obtained in rats that were exposed to nicotine as adults; i.e. in the minimum and median groups the percent liquid rats drank from the nicotine bottle decreased when the nicotine concentration was increased from 10 to 20 mg/L after the initial two weeks (data not shown).

3.6. The effect of nicotine on body weight in adult rats

To study the effect of oral nicotine self administration on weight gain, difference in body weight over six weeks of nicotine exposure was evaluated in adult rats. Initially an ANOVA was performed with sex and nicotine exposure (nicotine, water) as between-subjects factors and weight gain over six weeks as the dependent variable. Both sex [$F(1, 42) = 15.89$, $p < 0.001$] and nicotine exposure [$F(1, 42) = 45.35$, $p < 0.001$] emerged as significant effects. Females had smaller weight gain than males and nicotine treated animals had smaller weight gain than water treated controls (Table 4). Separate ANOVAs were performed for male and female rats with difference in body weight as the dependent variable and nicotine preference (Ward groups) as the between subjects factor; post-hoc tests were performed to depict differences between the groups. In both females and males, there were significant differences between the weight gain of rats with different nicotine preferences and subsequently nicotine consumption: [Females: $F(3,19) = 11.36$, Males: $F(3,22) = 8.50$; $p < 0.001$ for both sexes]. However, the effect on females was more pronounced than males (Table 5).

3.7. Effect of saccharine on oral nicotine self administration

3.7.1. Nicotine intake—To study the effect of saccharine on nicotine self administration, average nicotine intake was evaluated in two weeks periods: 1&2 (10mg/L nicotine, without saccharine), 3&4 (20mg/L nicotine, without saccharine), 5&6 (20mg/L nicotine, with saccharine), 7&8 (20mg/L nicotine, without saccharine). Initially a repeated measures ANOVA was performed with sex (female, male) as the between subjects, treatment in two week periods (1&2, 3&4, 5&6, 7&8) as the within subjects factor; average nicotine intake for each two week period was the dependent variable. Treatments emerged as a significant main effect [$F(3, 108) = 12.967$, $p < 0.001$] and interacted with sex [$F(3, 108) = 4.427$, $p < 0.01$]. As depicted in Figure 8 nicotine intake varied over weeks and this was more pronounced in males.

3.7.2. Individual differences—Ward analyses was applied to the data to see if rats in this experiment were divided into minimum, median and maximum preferring groups based on the average nicotine consumption over two week periods. As depicted in Table 6, rats in this experiment were also divided into the three groups mentioned ($p < 0.001$ in all cases). An ANOVA with sex, treatment, and Ward group (minimum, median, maximum) as the nicotine intake as the dependent variable revealed significant main effects of Treatment [$F(3, 154) = 91.603$, $p < 0.001$] and Ward group [$F(2, 154) = 303.201$, $p < 0.001$], and interactions between Sex $\times$ Treatment [$F(3, 154) = 11.967$, $p < 0.001$], Treatment $\times$ Ward group [$F(6, 154) = 6.273$, $p < 0.001$], and Sex $\times$ Treatment $\times$ Ward group [$F(6, 154) = 3.213$, $p < 0.01$]. Briefly, sex differences were not significant, but nicotine consumption among different Ward groups was significantly different and the addition of saccharine increased nicotine consumption. Post hoc Bonferroni test results are given in Table 6. However, Chi-Square Tests (McNemar-Bowker Test) did not depict any significant difference between any 2-week period regarding the number of animals in each Ward group.

3.7.3. Weight gain and total liquid intake—The total weight gain over 8 weeks was 20.95 ± 2.79 and 26.06 ± 4.87 for female and male rats, respectively. There was no sex difference in weight gain (t-test, $p > 0.05$). The effect of saccharine on total liquid intake was evaluated by repeated measures ANOVA with weeks as the within subjects factor and sex as

the between subjects factor; significant main effects of weeks [$F(7, 203)=28.105$, $p<0.001$] and sex [$F(1, 29)=13.585$, $p<0.001$] emerged. Liquid intake varied over weeks and male rats drank more liquid than female rats; however there was no interaction (Table 7). Post hoc tests indicated that the addition of saccharine increased total liquid intake significantly.

4. DISCUSSION

The studies described in this paper were designed to address the following questions:

- Does oral intake change with age?
- Does exposure to nicotine in the drinking water as an adolescent affect oral intake of nicotine as an adult?
- Does sex influence oral nicotine intake?
- Is oral self administration a stable behavior?
- Does the addition of saccharine alter nicotine intake?

The results of our experiments demonstrate that nicotine concentration, age of initiation, duration of access, and sex influence oral nicotine intake as measured in a continuous access 2-bottle choice test. We also found that the addition of saccharine to the drinking solutions increased overall intake of both the vehicle and nicotine-containing solutions, but saccharine did not seem to alter the effects of the other factors that were studied.

We found that the volume of nicotine-containing solution consumed decreased with an increase in nicotine concentration. This finding suggests that the rats altered their behavior to regulate nicotine intake. Other laboratories have also shown that rats (Adriani et al., 2002b; Biondolillo et al., 2008) and mice (Lee et al., 2004; Robinson et al., 1996) can discriminate nicotine and regulate their intake when the nicotine dose in the drinking solution is varied. However, the alteration in behavior does not prove that the animals were drinking nicotine to obtain a behavioral effect, especially a reinforcing effect. Le Houezec (Le Houezec et al., 1989) argued over 20 years ago that oral nicotine self-administration cannot be used to evaluate the reinforcing effects of nicotine because rodents do not prefer nicotine-containing solutions over water. However, our results show that most of the rats in the maximum preferring groups preferred nicotine over water and drank more than 50%, and as high as 93%, of the liquid from the nicotine side with or without saccharine.

We saw dose-dependent effects on weight in both sexes, but males seemed to be less sensitive: significant reductions in weight gain were seen in all three intake groups in female rats but only in the two higher intake groups in males. The finding that the weight gain of rats that were treated with nicotine was significantly less than the weight gain seen in control rats supports the assertion that orally-supplied nicotine exerted a systemic effect.

A literature is slowly developing that argues that factors that occur after nicotine has been absorbed influence nicotine intake and that these factors modulate the reinforcing effects of nicotine. Siu et al. (Siu et al., 2006) found that C57BL/6- St/bJ F2 hybrid mouse crosses with higher levels of the enzyme that metabolizes nicotine (CYP2A5) drink more nicotine than low activity mice, indicating that nicotine intake is influenced by factors that are acting after nicotine has been absorbed from the gastrointestinal tract. Two recent studies that used F2 hybrid mice derived from high-preferring C57BL6 and low-preferring A/J (Butt et al., 2005) or C3H (Li et al., 2007) mice determined that nicotine intake is influenced by polymorphisms in several, perhaps many genes and that one of these is the gene that codes for the mouse $\alpha 4$ nicotinic cholinergic receptor subunit (Chrna4) gene. These results agree

with others that support the assertion that nAChR subunit genes mediate sensitivity to nicotine (Butt et al., 2005; Li et al., 2007; Portugal and Gould, 2008; Robinson et al., 1996).

We used outbred Sprague Dawley rats of both sexes in an attempt to determine whether genetic factors influence the parameters that we measure. Marked individual differences in intake were detected in every experiment (Figure 5). These individual differences were seen in adolescent (intake varied between 1.5–12.0 mg/kg in the two experiments that used adolescent rats) as well as adult rats, and in experiments with or without the saccharine added to oral nicotine/water.

4.1. Does oral nicotine intake change with age?

Several of our experiments demonstrated that oral nicotine intake changes with age. Rats which had free access to nicotine consumed more nicotine when they were exposed at five weeks of age than when they were exposed at 10 or 17 weeks of age. Our finding is in agreement with the reported findings that both male and female adolescent rats (Maehler et al., 2000) and mice (Klein et al., 2004) consumed more nicotine (2-bottle, free choice, oral) than adults, that female adolescent Sprague-Dawley rats self administered significantly higher doses of nicotine intravenously compared to adults (Levin et al., 2003), that i.v. nicotine self administration in adolescent rats is initially greater than in adults, but the amount decreased over weeks and by the fourth week, was not different from adults (Levin et al., 2007). The finding that the rewarding effects of nicotine in the conditioned place preference test is greater in adolescent than adult male (Kota et al., 2007) and female (Kota et al., 2008) mice adds further support for the postulate that adolescent rodents are more sensitive to the reinforcing effects of nicotine. In contrast, Shram et al (Shram et al., 2008) concluded that nicotine is less reinforcing in adolescent compared with adult rats based on results obtained in an i.v. nicotine self administration study that used different reinforcement schedules, followed by tests for extinction and reinstatement of nicotine seeking in adolescent and adult male rats. Rats exposed to nicotine as adults displayed greater resistance to extinction of nicotine taking behavior compared to rats exposed to nicotine as adolescents. Reinstatement, on the other hand, was independent of the age of first exposure.

4.2. Does exposure to nicotine as an adolescent affect intake as an adult?

The nicotine doses consumed by adult rats that had not been pre-exposed were similar to those consumed by adolescent rats (average doses ranged from 0.5–12 mg/g). However, pre-exposure to nicotine during adolescence affected intake. Adult rats from the continuous and interrupted treatment experiment consumed nearly 2 or 3 mg/kg, respectively (Figure 4) whereas adult rats that had not been treated with nicotine as adolescents (i.e. adult only testing) consumed approximately 4–5 mg/g each week. Our results compare favorably with two studies from the Levin laboratory (Levin et al., 2007; Levin et al., 2003) that evaluated i.v. nicotine self-administration of adolescent and adult female (Levin et al., 2003) and male (Levin et al., 2007) Sprague Dawley rats. Adolescent rats of both sexes self-administered more nicotine than did adults. Nicotine self-administration remained higher than adult levels in female rats that had been tested as adolescents (Levin et al., 2003) but males showed a decrease in the behavior so that adolescent-exposed rats were not different from male rats that were tested for the first time as young adults. The results of our experiment differ from those obtained by Adriani et al (Adriani et al., 2003) who found that the i.v. self-administration of nicotine was significantly greater in rats that had been injected (s.c.) with nicotine for 10 days as adolescents five weeks before the rats were subjected to i.v. nicotine self administration. The differences between the Adriani et al study and the present one may arise because the route of nicotine administration and the duration of exposure are different. Nicotine injections unquestionably produce a different pharmacokinetic profile (higher peak

concentrations) than does oral intake which could produce effects regulated by a different mechanism(s) of action.

Adolescent nicotine exposure seems to produce long-lasting neurochemical effects that persist into adulthood in rodents (reviewed in (Izenwasser, 2005)). Nicotine exposure during 8–16 postnatal days resulted in long lasting upregulation of nAChRs and converted low affinity binding sites to high affinity binding sites in adult rat brain (Miao et al., 1998; Nordberg et al., 1991). Moreover, Slotkin et al (Slotkin, 2002) found different patterns of nAChR upregulation across brain regions elicited by nicotine administration in adolescent and adult rats.

4.3. Does sex influence oral nicotine intake?

Sex did not emerge as a main effect in ANOVAs. This result agrees with the report of Maehler et al (Maehler et al., 2000) who did not observe significant sex differences in oral nicotine self administration in rats. However, we did detect subtle sex differences in post-hoc tests, especially regarding the average nicotine consumption in different Ward groups during adulthood. When adult rats were divided into Ward groups the consumption of females in the median groups was higher than males and consumption of males in the maximum groups was higher than females (Table 1). Marshall et al. (Marshall et al., 2003) used a similar protocol in adolescent and adult Sprague Dawley male and female rats and found that nicotine consumption was higher in adolescents than adults.

Another interesting finding of the current study was the change in the pattern of nicotine consumption (reflected by the preference according to the Ward groups rather than absolute nicotine consumption) as rats progress from adolescence to adulthood; the change in pattern of nicotine consumption is more pronounced when there is an interval between adolescent and adult exposure and while females reduce their preference, males increase it, notwithstanding the fact that females are more persistent in their preference pattern.

The rats used in these studies were exposed to nicotine chronically. This may be important given that some of the changes induced by chronic nicotine exposure are sexually dimorphic (reviewed by (Slotkin, 2002)). When adolescent male and female rats received nicotine through implanted minipumps (Slotkin, 2002) or male and female mice were exposed to the two-bottle free choice nicotine condition (Klein et al., 2004), females consumed more nicotine than males but the serum cotinine levels were similar, suggesting sex differences in nicotine metabolism and related pharmacokinetic processes.

4.4. Is oral self administration a stable behavior?

The average nicotine intake decreased over time in the continuous access experiment. Adolescent rats drank, on average, approximately 5–6 mg/kg each week, but the average dose decreased to less than 2 mg/kg, on average, when the rats were 14 weeks old. Virtually all of the previously published studies that measured oral nicotine intake in rodents evaluated nicotine intake for 1–2 weeks. In the current study, we evaluated the nicotine consumption patterns of rats starting at adolescence and continuing through adulthood. Some rats which had access to nicotine as adolescents and also as adults (continuous and interrupted regimens) had a persistent pattern of consumption while others did not. In the continuous regimen, 57% of the female rats and 47% of the male rats were in the same Ward group as adolescents and adults, in other words, they showed the same pattern of nicotine preference as adolescents and adults. In the interrupted regimen, these ratios were 42% and 17% for females and males, respectively (Table 3). Figure 6 shows the persistence of nicotine preference as rats proceed from adolescence to adulthood. As depicted in the figure, the behavior is very stable in female minimum preferring rats: 85% of the female rats that

were designated as minimum consumers as adolescents were still minimum consumers after 23 weeks of testing, while this ratio was only 41% in male rats (minimum preferring as adolescents and minimum preferring as adults). Considering that all the animals were under identical conditions throughout the experiment, our findings suggest that genetic factors may be more pronounced in female rats than male rats and that environmental influence may have greater impact when nicotine exposure is interrupted between adolescence and adulthood.

The Ward cluster analyses divided the rats into three subgroups in every experiment. However, the consumption pattern of some rats in the “median” group was not as consistent as those in the “minimum” and “maximum” groups (Table 2). These results agree remarkably well with those obtained by Maehler et al (Maehler et al., 2000) in a study that measured voluntary nicotine consumption in male and female Sprague-Dawley rats using the 2-bottle free-choice method. Maehler et al (2000) also found that adult rats could be divided into three groups based on their nicotine consumption, that minimum and maximum nicotine preferring rats have a consistent pattern, and that those in the median group show greater variability.

4.5. Does saccharine alter nicotine intake?

The findings that genetic modulations and drug pretreatments alter oral nicotine intake do not eliminate the possibility that taste factors will influence oral intake of nicotine, as well. Nicotine is described as bitter tasting (Iwasaki and Sato, 1981) which makes sense given that nicotine elicits responses in chorda tympani neurons that are sensitive to classical bitter tastants such as quinine (Carstens et al., 1998; Dahl et al., 1997; Lyall et al., 2007). Findings that indicate the potential importance of taste factors in regulating smoking include: 1) ability to taste phenylthiocarbamide (PTC) seems to affect smoking status (incidence is higher in non-tasters) in Native Americans (Enoch et al., 2001); 2) female Caucasian PTC non-tasters show more severe signs of dependence than non-tasters (Snedecor et al., 2006) and; 3) polymorphisms in two bitter taste receptors (TASR16 and TAS2R38) that promote reduced ability to taste bitter substances are positively associated with nicotine dependence in African American women (Mangold et al., 2008). These results argue that genetic mutations that result in a reduced ability to taste bitter substances serve to increase vulnerability to tobacco addiction. Additionally, Tomassini et al (Tomassini et al., 2007) studied the effect of oral nicotine administration (3 weeks) on taste buds in adolescent and adult rats; the results suggest significant alteration of the properties of taste buds in rat fungiform papillae. Subsequently long term oral nicotine administration without saccharine to mask the bitter taste of nicotine may have modulatory effects on taste.

All of our experiments were conducted with saccharine added to both nicotine and water bottles. This procedure was used in an attempt to mask the bitter taste of nicotine. We tested the potential effects of saccharine on oral nicotine intake in an experiment that compared nicotine intake of rats with and without saccharine added to the drinking solutions. As depicted in figure 8, adult rats drank nicotine without saccharine added and showed individual variation in their preference for nicotine; saccharine increased average nicotine (~1.7 mg/kg/week) and total liquid (~70 ml/week) intake. These results closely parallel results obtained using inbred mouse strains. Robinson et al. (Robinson et al., 1996) used nicotine solutions with or without saccharine added and observed an increase in total liquid intake without an alteration in the ratio of nicotine/water.

5. CONCLUSIONS

The present study supports findings from other laboratories that presenting rats with a free choice of oral nicotine is a reliable method of self administration. Although addition of

saccharine to the nicotine/water solutions increases nicotine intake, rats drink nicotine solution without saccharine and based on their individual preference level, prefer nicotine over water. In this paradigm, the behavior of the rats changes with time; age, sex and previous exposure influence nicotine intake significantly. In all of the experiments (adolescent or adult, with or without previous nicotine exposure, with or without saccharine added to drinking solutions) rats could be divided in to three subgroups based on their nicotine consumption patterns: minimum, median and maximum nicotine preferring. In view of the more stable consumption pattern of female rats as they progress from adolescence to adulthood, genetic factors appear to play a stronger role in females and environmental factors in males. Overall, our findings show that there are individual differences in nicotine preference and intake in Sprague Dawley rats which vary according to age, sex and previous exposure.

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Figure 1. Oral nicotine self administration protocols

Rats were exposed to a free choice of oral nicotine at different ages and for different durations. Rats were divided into minimum, median and maximum nicotine preferring groups using the Ward analysis, based on their weekly nicotine consumption for 6-week durations. In one experiment the effect of saccharine was evaluated (Adults, saccharine); in this experiment, the duration was eight weeks. The horizontal cylinders show the five sets of animals in 4 experiments that self administered oral nicotine through different protocols..

Figure 2. Time course of nicotine consumption

Rats (n=62, 33 F) had free access to a choice of nicotine or water provided in two bottles continuously, 24 hours/day, for 23 weeks, starting at five weeks of age. Each point shows average weekly nicotine consumption $\pm$ SEM. Sex difference, * $p < 0.05$ (t-test)

Figure 3. Time course of nicotine consumption over six weeks in rats with different ages of onset
Rats had free access to a choice of nicotine or water provided in two bottles continuously, 24 hours/day. The figure shows average weekly nicotine consumption for six weeks in rats with different ages of onset of nicotine exposure: adolescent (5 weeks), young adult (10 weeks), adult (17 weeks). Male and female data are pooled. Each point shows average weekly nicotine consumption $\pm$ SEM.
Different from adolescent ** $p < 0.005$, * $p < 0.05$; different from adult ‡ $p < 0.05$ (Post hoc Bonferroni tests)

Figure 4. Nicotine consumption in adult rats with or without previous exposure

The bars show average weekly nicotine consumption in adult rats over six weeks of exposure. Continuous: Rats had continuous exposure to oral nicotine starting at 5 weeks of age. The presented data is derived from weeks 22–28.

Interrupted: Rats were exposed to oral nicotine at 5 weeks of age for 6 weeks, and then had an interval of 9 weeks. The presented data is derived from weeks 19–25.

Adult exposure: Rats were exposed to oral nicotine only as adults (17 weeks). The presented data is derived from weeks 17–23.

Post hoc Bonferroni tests (males and females merged): *** $p < 0.001$, different from the other two groups.

Figure 5. Individual differences in average weekly nicotine consumption over six weeks of oral nicotine self administration in four different regimens of nicotine exposure

Vertical axis shows the number of rats. The polynomial trend lines (females: broken, males: solid) are provided to show different patterns in individual variability in nicotine consumption during different experimental conditions: Adolescent rats from two experiments combined (top left hand, data derived from 5–11 weeks of age), adult rats exposed to nicotine at 17 weeks of age without any previous exposure (top right hand, data derived from 17–23 weeks of age), adult rats that had continuous exposure starting at adolescence (bottom left hand, data derived from 22–28 weeks of age), and adult rats which were exposed to nicotine for 6 weeks starting at adolescence and were exposed again as adults following a 9 week interval (bottom right hand, data derived from 19–25 weeks of age). Please note that, as mentioned in the methods section, the total number of rats are different in each case (y-axis) and that the x-axis in the bottom panels are different from those on the top panels.

Figure 6. The change in the trend of nicotine preference between adolescence and adulthood
 This figure is intended to show and compare the change in the nicotine preference of rats as they grow from adolescence into adulthood. Rats were exposed to a free choice of oral nicotine and water starting at adolescence and continuing exposure into adulthood. In the continuous regimen, there was no interruption of nicotine exposure while in the interrupted regimen, there was an interval of 10 weeks between adolescent and adult exposure. Preferences were based on Ward analyses with average nicotine intake as the dependent variable, measured for 6 weeks exposure periods as adolescents and adults. The Ward grouping as adolescents is provided on top of each pie graph (minimum, median or maximum in white, gray or black backgrounds, respectively). The pie graphs show the percentage of the rats in the Ward groups as adults; the color coding is the same (i.e. white=minimum, gray= median, and black= maximum nicotine preferring rats). The percentages show the rats whose preference did not change as they grew older. The figures should be read as follows: The pie graph on the right hand side of the 1A group indicates that of the female rats which had minimum nicotine preference as adolescents, 84.6% were still minimum preferring as adults. The graph on the right hand side of the 1B group,

indicates that of the male rats which had minimum nicotine preference as adolescents, 41.2% were still minimum preferring as adults; however most of the rats had increased their preference and are now in the median preferring group as adults.

Figure 7. Regulation of nicotine intake

The amount of liquid rats drank from the nicotine containing bottle (in control animals, water from the bottle on the same side) was measured over a period of six weeks. The bars show average weekly liquid the rats drank in two week periods (1–2, 3–4, 5–6) $\pm$ SEM. Nicotine concentration in drinking water was 10 mg/L during the 1–2 weeks and 20 mg/L during the 3–4 and 5–6 weeks. The change in concentration is indicated by the symbol

Different from 1–2*** $p < 0.001$, ** $p < 0.005$, * $p < 0.05$

Figure 8. The effect of saccharine on nicotine intake

The effect of saccharine on nicotine intake was evaluated by giving the rats nicotine/water without saccharine, then adding saccharine and then removing saccharine. On weeks 1&2 rats received an oral choice of nicotine (10 mg/L) or water, on weeks 3&4, nicotine concentration was increased (20 mg/L), on weeks 5&6 saccharine was added to both bottles (20 mg/L nicotine, 2% saccharine), on weeks 7&8 saccharine was removed and the conditions were the same as week 3&4.

In females, there were no significant differences in nicotine intake between the 2-week periods. In males, nicotine intake during weeks 1&2 and 7&8 was lower than in weeks 5&6 (saccharine added): Different from 5+6, *** $p < 0.001$; Different from 1+2, ** $p < 0.01$.

Table 1

The average weekly nicotine consumption (over 6 weeks) in the subgroups of rats exposed to oral nicotine in 4 different regimens.

	Adolescent				Adult			
	minimum	median	maximum	minimum	median	maximum	minimum	maximum
F								
cont	3.01 ± 0.08	5.10 ± 0.3	8.27 ± 0.11	0.88 ± 0.04	♂♂ 1.39 ± 0.03	♂♂ 2.66 ± 0.10		
Inter	3.07 ± 0.15	4.94 ± 0.13	6.89 ± 0.28	1.81 ± 0.10**	♂♂ 3.51 ± 0.16**	4.94 ± 0.15 *		
10 w				1.82 ± 0.43**	4.10 ± 0.23 **	7.21 ± 0.83 **		
17 w				1.45 ± 0.13 *	3.44 ± 0.17 **	7.62 ± 0.59 **, ##		
M								
cont	3.35 ± 0.20	5.55 ± 0.17	8.43 ± 0.65	0.76 ± 0.07	2.16 ± 0.10	3.87 ± 0.31		
Inter	3.69 ± 0.09	5.61 ± 0.04	9.26 ± 0.27	1.24 ± 0.12	2.28 ± 0.12 *	3.70 ± 0.24		
10 w				1.89 ± 0.07	3.15 ± 0.14 **, #	5.39 ± 0.19 #		
17 w				2.55 ± 0.34 **	6.78 ± 0.25 **, ##, #	9.72 ± 0.50 **, ##, ##		

F: Female, M: Male, cont: Continuous regimen, inter: Interrupted regimen, 10 w: Onset at 10 weeks of age, 17 w: Onset at 17 weeks of age

Bonferroni tests (same sex, adults)

Different from continuous (**; p<0.001, *: p<0.01)

Different from interrupted (###; p<0.001, #: p<0.01)

Different from 2m (##; p<0.001)

t-tests (same regimen, same rank)

Different from males (♂♂; p<0.001, ♂♂; p<0.05)

No differences were depicted (t- tests, p>0.05) between the nicotine consumption levels of adolescent rats in the same preference groups (same sex)

Table 2
Data from individual rats with different preferences for nicotine over six weeks starting from the initial exposure to oral nicotine self administration

The data from rats with variable nicotine consumption across weeks are shown in *Italic* font, shaded.

		Weekly nicotine consumption (mg/kg)							
		1	2	3	4	5	6	AVERAGE	
Females	Adolescent	minimum	2.00	1.54	1.25	1.82	0.83	1.43	1.48
		median	4.17	4.29	2.86	2.50	2.00	3.33	3.19
		maximum	5.35	6.43	6.25	10.53	11.30	6.96	7.80
		variable	6.32	1.36	14.62	0.71	2.00	0.61	4.27
	Adult	minimum	0.30	2.42	0.38	0.62	0.82	1.56	1.01
		median	1.37	2.21	4.14	1.20	3.72	3.48	2.69
		maximum	6.16	7.00	6.50	15.87	10.32	6.62	8.75
		variable	1.91	7.25	0.92	9.43	0.87	0.41	3.47
Males	Adolescent	minimum	1.75	2.71	1.34	2.31	1.43	0.56	1.68
		median	5.00	3.33	5.33	2.33	4.83	2.57	3.90
		maximum	10.83	10.83	3.64	13.33	4.00	10.00	8.44
		variable	12.50	0.43	5.38	0.59	1.00	1.43	3.56
	Adult	minimum	2.12	0.35	0.43	0.80	1.76	0.29	0.96
		median	1.37	2.21	4.14	1.20	3.72	3.48	2.69
		maximum	7.42	3.40	14.21	13.27	8.78	9.45	9.42
		variable	1.40	0.19	14.31	1.86	1.18	3.33	3.71

Table 3
The number and percentage of rats which displayed a trend of stability or change in their preference patterns (based on Ward groups)

“Increased” means that a rat that was in the minimum (or median) group as an adolescent is median or maximum (or maximum) nicotine preferring as an adult.

“Decreased” means that a rat that was in the maximum (or median) group as an adolescent is median or minimum (or minimum) nicotine preferring as an adult.

“Same level” means that a rat that the nicotine preference of the adolescent rat has not changed as the rat grew and became an adult; the rat was in the minimum (or median, or maximum) group as an adolescent and is in the same nicotine preferring group as an adult.

	Continuous exposure		Interrupted exposure	
	Females	Males	Females	Males
Increased	5 (14 %)	11 (37 %)	9 (21 %)	14 (58 %)
Decreased	10 (29 %)	5 (16 %)	16 (37 %)	6 (25 %)
Same level	20 (57 %)	14 (47 %)	18 (42 %)	4 (17 %)
Total	35 (100 %)	30 (100 %)	43 (100 %)	24 (100 %)

Table 4

Weight gain in young adult rats during six weeks of treatment

Sex	Weight gain (g)	
	Nicotine treated	Control
Female	6.25 ± 6.99***, ‡‡‡	55.13 ± 7.19 [‡]
Male	34.07 ± 6.26***	88.38 ± 4.02

Average weight gain ± SEM

Different from control (same sex): *** p<0.001 (t-test)

Different from male (same treatment): [‡] p<0.05, ‡‡‡ p<0.001 (t-test)

Table 5
Difference in weight gain between rats with different nicotine consumption patterns

Young adult rats with different nicotine preferences (minimum, median, maximum) and control animals that received water from both bottles (Average + SEM). n=27 (12F) for nicotine/water treated and n=16 (8F) for water/water treated rats; 3–8 rats in each group.

	Females	Males
Minimum	10.25 ± 4.09 *	60 ± 20.82
Medium	−2.6 ± 9.1 ***	23.14 ± 10.89 ††
Maximum	15.67 ± 12.73 *	33.8 ± 15.97 †
Control	55.13 ± 7.19	88.38 ± 4.02

Different from female control * p<0.05, ** p<0.01, *** p<0.001.

Different from male control † p<0.01, †† p<0.001

Table 6

Average nicotine intake in minimum, median and maximum nicotine preferring rats exposed to a nicotine/water choice without and with saccharine added.

Post hoc Bonferroni tests, following ANOVA (details provided in the results section), showed significant differences ($p < 0.001$) between the Ward groups, and some treatments: Weeks 3&4, 5&6 and 7&8 were different from weeks 1&2 ($p < 0.001$) and weeks 1&2, 3&4 and 7&8 were different from weeks 5&6 ($p < 0.001$). Please note that weeks 3&4 and 7&8 were not different from each other ($p > 0.05$) showing clearly that adding saccharine did not affect nicotine intake and that the behavior was stable.

	weeks	Treatment	minimum	median	maximum
FEMALE	1&2	Nicotine (10 mg/L) no saccharine	2.32 ± 0.28	3.93 ± 0.12	5.37 ± 0.19
	3&4	Nicotine (20 mg/L) no saccharine	1.96 ± 0.18	4.10 ± 0.36	6.82 ± 0.43
	5&6	Nicotine (20 mg/L) with saccharine	3.42 ± 0.40	7.49 ± 0.26	11.69 ± 0.62
	7&8	Nicotine (20 mg/L) no saccharine	2.97 ± 0.26	6.26 ± 0.28	8.86 ± 0.91
MALE	1&2	Nicotine (10 mg/L) no saccharine	0.99 ± 0.27	3.46 ± 0.20	5.42 ± 0.57
	3&4	Nicotine (20 mg/L) no saccharine	2.54 ± 0.59	6.12 ± 0.30	8.20 ± 0.13
	5&6	Nicotine (20 mg/L) with saccharine	4.61 ± 0.56	7.61 ± 0.33	11.20 ± 0.57
	7&8	Nicotine (20 mg/L) no saccharine	2.53 ± 0.17	4.60 ± 0.38	6.32 ± 0.39

Table 7
Change in liquid intake over weeks in rats exposed to an oral nicotine/water choice with and without saccharine

One Way ANOVA performed for females and males separately revealed significant differences in total liquid intake over 8 weeks: F(7, 156)=18,417 and F(7, 146)=9,894 for females and males, respectively, $p<0.001$ for both sexes.

Weeks	1	2	3	4	5	6	7	8
Females	106 ± 5	138 ± 10	143 ± 6**	153 ± 6**	156 ± 8**	230 ± 14***	134 ± 4	166 ± 10**
Males	136 ± 13	145 ± 12	156 ± 11	167 ± 5	192 ± 11**	256 ± 13***	145 ± 12	185 ± 20

Different from all of the other weeks: *** $p<0.001$; Different from week 1: ** $p<0.01$ (Post hoc Bonferroni tests)