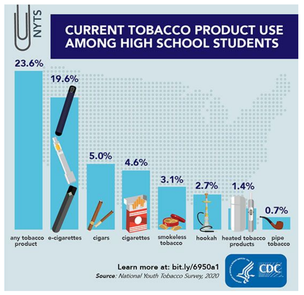
THE INFLUENCE OF TOBACCO FLAVORING ON ORAL NICOTINE CONSUMPTION IN C57BL/6J MICE WITH RESPECT TO AGE AND SEX

Yumna Rahman[1], Lois Akinola[1], Olivia Ondo[1], M. Imad Damaj[1]

[1]Department of Pharmacology and Toxicology, VCU School of Medicine

Introduction



The World Health Organization reports over 8 million deaths annually from tobacco use. Nicotine, the primary psychoactive constituent in tobacco, plays a significant role in the initiation and maintenance of tobacco addiction. The presence of flavorants in nicotine products has contributed significantly to their continued use. Flavorants are often used to improve the taste and perceived odor of these products, however, their role in the maintenance of nicotine dependence and the pertinent mechanisms are poorly understood.

They are not only used in traditional cigarettes, but in other smokeless tobacco products including electronic cigarettes. E-cigarettes are appealing, especially to adolescents and females, because of the wide range of available flavors. Prior research shows that adolescents are more susceptible to drug use and abuse than adults and because the brain is under active development during adolescence, drug use is more likely to have an impact on the underdeveloped neural network of the brain. According to the National Tobacco Youth Survey, in 2019, 53.3% of U.S. high school students and 24.3% of U.S. middle school students have tried tobacco products, with e-cigarettes reportedly being more popular than combustible cigarettes.

Likewise studies on nicotine dependence demonstrate sex difference in sensitivity to the effects of nicotine and in the use of flavored nicotine products. While the prevalence rates for tobacco use are generally higher in men than women, women are one of the biggest targets of the tobacco industry. They are also at greater risk for developing smoking-related diseases and have poorer cessation outcomes compared to men.

Accumulating evidence suggests that in addition to increasing the appeal of tobacco products, flavorants may exert pharmacological effects beyond their function as odorants. There has been a prominent focus on menthol, whereas, fewer studies have focused on tobacco flavors.

Objective and Hypothesis

1. To investigate the impact of tobacco flavoring on oral nicotine consumption using the two-bottle choice (2BC) paradigm

1. Investigate the impact of age and sex in their interactions

We hypothesized that the addition of tobacco flavoring will increase oral nicotine consumption and that adolescent C57BL/6J (B6J) mice will display a higher preference and intake compared to adult B6J mice.

Methods

Animals

- Naive adolescent (PND 21) and adult (PND 60), male and female C57BL/6J (B6J) mice
- n=10 per sex for each cohort

Two-Bottle Choice (2BC) Test (pictured below)

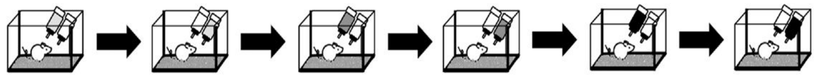
- First, animals are given two bottles of DI water for one week before being put in their respective cohorts depending on what test solution was being administered
- Daily fluid intake was recorded for each tube, and the tube positions were switched following recording to prevent the development of a side preference

Solution Preparation

- Test solution contained either diluted tobacco flavor concentrate alone, nicotine alone, or a combination of nicotine and tobacco flavor concentrate
- For nicotine-only solution, nicotine base (Sigma Aldrich) was diluted in DI water
- Tobacco solutions were prepared by diluting liquid tobacco flavor concentrate purchased from a commercial vendor (Avail Vapor) and for the combination, nicotine base and tobacco flavor concentrate were both diluted in DI water.

Data Analysis

- Intake was calculated as mg of drug per kg of body weight per day
- Preference was calculated as volume of solution intake as percentage of total fluid intake
- Data was analyzed with GraphPad Prism software and expressed as the mean ± S.E.M
- Concentration-response studies were performed using a within-subject design Statistical analysis was conducted using a three-way repeated-measures analysis of variance test followed by the appropriate post hoc test where significant.



Adult B6J Results

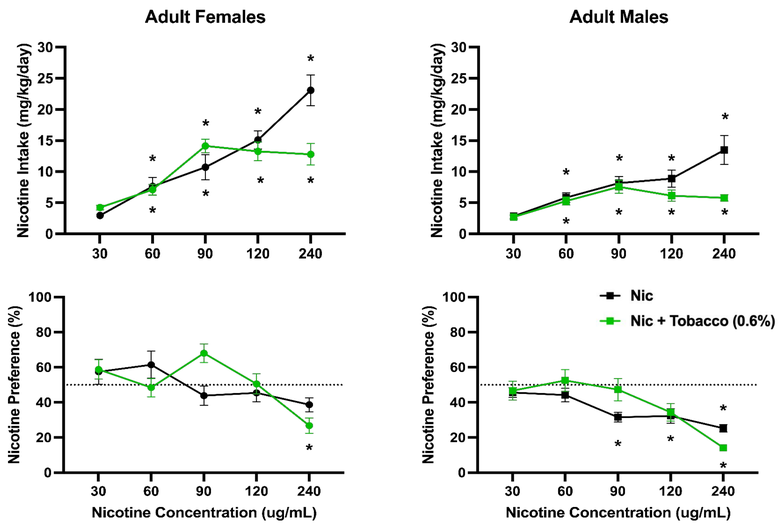


Figure 3: Nicotine preference and intake was measured at increasingly higher concentrations of nicotine solution, with a male and female cohort being given 0.6% tobacco flavoring in addition to nicotine.

In adult males and females, we observed a decrease in nicotine intake at high doses with the addition of tobacco flavoring. The trend in preference does not differ significantly overall between the two test solutions in adult males or females.
***Denotes significant difference compared to the 30 ug/mL concentration, p<0.05.**

Tobacco Flavoring Dose-Response Curve

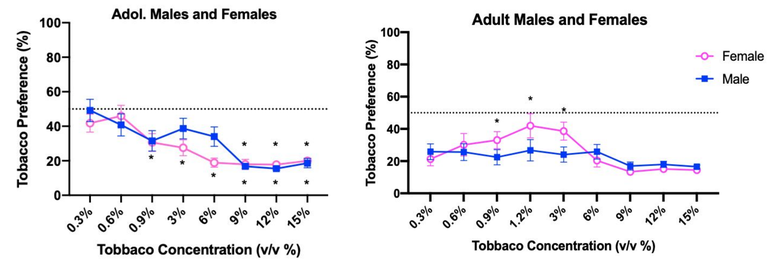


Figure 1: Tobacco preference was recorded at increasingly high concentrations. There is a significant increase in preference at the 0.9, 1.2, and 3% concentrations in adult females. Adolescent data reveals a trend of decreasing preference as tobacco concentration is increased. There is a lack of overall effect of sex. *Denotes significance as compared to the 0.3% concentration, p<0.05.

Adolescent B6J Results

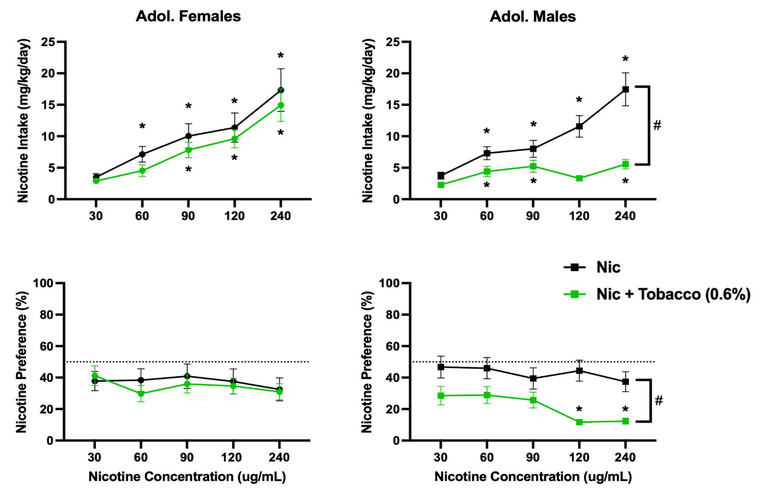


Figure 2: Nicotine preference and intake was measured at increasingly higher concentrations of nicotine solution with a male and female cohort being given 0.6% tobacco flavoring in addition to nicotine.

In adolescent males, we observed a significant decrease overall in preference and intake of nicotine with the addition of tobacco, while in females, no such difference was noted between the test solution types.
***Denotes significant difference compared to the 30 ug/mL concentration. #Denotes an overall significant difference between the nicotine and nicotine+tobacco cohorts, p<0.05.**

Discussion and Future Directions

We demonstrate that:

- Tobacco row flavoring from Avail Vapors did not significantly increase nicotine consumption at the single concentration (0.6%) tested.
- Overall, adult B6J mice had greater preference and intake for nicotine + tobacco flavoring compared to adolescent mice. In both adolescent and adult male animals, tobacco appeared to reduce nicotine intake rather than increase it as hypothesized.
- Data revealed significant effects of sex, where female mice on average showed significant increases in nicotine consumption compared to males.
- Future directions will test higher concentrations of tobacco flavoring to determine if there was a concentration-limiting effect and to determine if the preferred concentration of tobacco flavoring can mask the gustatory effects of nicotine at adversely high concentrations.

Acknowledgements and References

I would like to sincerely thank Lois Akinola for training me on this paradigm and assisting me in all of my questions along the way, and Dr. Damaj for his expert guidance throughout this project.

This research was funded by the National Institute on Drug Abuse and the Center for Tobacco Products of the U.S. FDA.

Bagdas, D., C. M. Diester, J. Riley, M. Carper, Y. Alkhlaif, D. Al Omari, … M. I. Damaj. 2019. Assessing nicotine dependence using an oral nicotine free-choice paradigm in mice. Neuropharmacology, 157, 107669. doi.org/10.1016/j.neuropharm.2019.107669