THE EFFECT OF EXERCISE ON NICOTINE CONSUMPTION IN ADULT C57BL/6J MICE

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The effect of exercise on nicotine consumption in adult C57BL/6J mice.

Thesis directed by Associate Professor Marissa A. Ehringer, Ph.D.

In the U.S., where nicotine is the most prevalent form of addiction, there are roughly 443,000 smoking related deaths per year and an additional 16 million who suffer from smoking related illness. Our lab has shown that exercise reduces alcohol consumption in mice. This study looked at the effect of exercise on nicotine consumption in adult mice. While the pilot study showed evidence for hedonic substitution, the main study had no significant results. Further research is needed to determine the effect of exercise on nicotine consumption.

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Introduction

Globally, tobacco use is responsible for more than five million deaths annually [1]. In the U.S., where nicotine is the most prevalent form of addiction [2], there are roughly 443,000 smoking related deaths per year and an additional 16 million who suffer from smoking related illness [1]. Furthermore, the transition from use to dependence occurs much more often in nicotine users than it does for alcohol, cannabis, or cocaine users [3]. Nicotinic acetylcholine receptors (nAChRs) are ligand gated ion channels that undergo a conformational change when the neurotransmitter acetylcholine (ACh) or alkaloid nicotine binds to the receptor, causing ions to flow into the cell. Chronic exposure to nicotine has been shown to upregulate nAChR expression, specifically increasing high-affinity (α4β2) nAChR nicotine binding in smokers [32]. Chronic self-administration of nicotine leads to tolerance caused by reduced receptor function due to desensitization. This desensitization can be linked to the addictive properties of nicotine and is associated with the physical symptoms of withdrawal [6].

There is well-documented evidence of alcohol and nicotine co-addiction in humans [5, 25-29]. Family and twin studies have provided increasing evidence of a genetic factor of addiction [4]. There is evidence that genetic factors contribute to roughly 50% (28%-84%) of the total variance contributing to smoking behaviors [5], and 50-60% of the variance contributing to alcohol behaviors [33]. Twin studies have shown a substantial genetic correlation between lifetime nicotine and alcohol dependence [33]. It has been shown that alcohol use disorders are associated with an increase in sensitivity to nicotine dependence symptoms, even in novel smokers

[7]. Many studies have established nicotinic acetylcholine receptors (nAChRs) as a common site of action among nicotine and alcohol. Ethanol potentiates the response of high-affinity nAChRs to both ACh and nicotine [8].

The mesolimbic dopaminergic reward pathway has been implicated in addictive behaviors including (but not limited to) the reward pathways of alcohol, nicotine, and exercise [8-11,18,19, 21-23]. This reward pathway is composed of dopaminergic neurons originating in the ventral tegmental area (VTA) and the substania nigra (SN) that project to the caudateputamen, nucleus accumbens (NAc), and the frontal cortex. Most drugs of abuse, including alcohol and nicotine are associated with an increase in dopamine (DA) release into the NAc by increasing the firing rate of DA neurons in the VTA [8,9,10]. Nicotine and alcohol show an additive effect on DA release in the NAc [10]. Additionally, repeated exposure to nicotine in the posterior increased the response of DA neurons to ethanol stimulation [11]. This shows that nicotine and alcohol use a similar reward pathway and could help to explain the co-addiction of nicotine and alcohol.

There have been several reports of a behavioral interaction between exercise and ethanol showing that access to exercise affects voluntary ethanol intake [12-19]. Our laboratory recently demonstrated that voluntary access to a running wheel reduces ethanol consumption and preference [17,18]. Given the high co-morbidity of nicotine and alcohol dependence, and the evidence for a similar reward pathway, we sought to investigate the effects of a running wheel on voluntary nicotine consumption and preference in adult mice.

Statement on Animal Care:

This study was conducted with the approval of the Institutional Animal Care and Use Committee at the University of Colorado, Boulder (Boulder, Colorado) following guidelines established by the Office of Laboratory Animal Welfare. All possible measures were taken to minimize animal discomfort.

Animals:

Animals were bred and housed at the Specific Pathogen Free facility, operated by the Institute for Behavioral Genetics at the University of Colorado, Boulder (Boulder, Colorado). Male and female C57BL/6J at least 60 days of age were used for these experiments. All animals were individually housed in 12x30.5x12.5 cm polycarbonate cages with or without access to free running or locked stainless steel wheels that are 108 mm in diameter. Each mouse was given a bedding square and given access standard chow and water *ad libitum* throughout the experiment. The mice were on a 12-hour light/dark cycle with light on at 7:00 AM.

Pilot Study

1. Methods

1.1 Behavioral paradigm

Mice were tested using a previously established paradigm that led to differences in ethanol consumption when given access to a free running wheel [17,18]. The four treatment conditions for the pilot study included:

1. Empty cage with access to one bottle of water and one bottle of 100 ug/ml nicotine solution two-bottle choice (n=10)
2. Running wheel with access to one bottle of water and one bottle of 100 ug/ml nicotine solution two-bottle choice (n=10)
3. Empty cage with access to one bottle of water and one bottle of 50 ug/ml nicotine for the first 4 days followed by 8 days of 100 ug/ml nicotine solution (n=5)
4. Running wheel with access to one bottle of water and one bottle of 50 ug/ml nicotine for the first 4 days followed by 8 days of 100 ug/ml nicotine solution (n=6)

The protocol lasted 15 days, including a three-day acclimation period during which all mice were given access to water only. Mice housed with a running wheel had 24-hour access to the wheel for all 15 days. Running distance, speed and time were recorded using a magnet and a bike speedometer. Any missing values (due to wheel or counter dysfunction) were computed by averaging the data from the day before and the day after. The side of the cage the bottles were on was switched every two days as to minimize placement preference. Individual consumption of water and nicotine were recorded daily. Body weights and food weights were recorded every 4 days on the same schedule.

1.2 Statistical analysis

All statistics were done using RStudio version 0.98.501 (www.rstudio.com). Measurements from days immediately following the weighing of mice (experimental days 1, 5, and 9) were excluded to control to behavioral differences that could be

caused by animal handling. A repeated measures ANOVA was used to identify group differences in nicotine consumption between mice given 100 ug/ml throughout the experiment and mice given graded nicotine 50-100 ug/ml. Within each concentration paradigm a two-way repeated measures ANOVA was used to identify differences in nicotine consumption (males vs. females and runners vs. non-runners). Within each sex a one-way repeated measures ANOVA was used to identify difference in nicotine consumption (runners vs. non-runners). One-way multiple measures ANOVAs were used to identify differences between runners' distance, speed and time (males vs. females and 100ug/ml paradigm vs. 50-100 ug/ml paradigm). Any missing values input by averaging the day before and the day after. The figures for consumption and preference for the pilot data were created using an average of experimental days 6-12 because drinking levels stabilized by experimental day 6.

2. Results

2.1 Voluntary running and nicotine consumption

Mice given access to a running wheel and 100 μg/mL nicotine solution (treatment 2) significantly consumed ($F=26.13$, $p<0.001$) and preferred ($F=47.85$, $p<0.001$) less nicotine than mice housed in an empty cage and given 100 μg/mL (treatment1) over the course of 12 days (Figures 1c and 2c). There was a main effect of sex on nicotine preference for mice given access to 100 ug/ml throughout the experiment ($F=15.96$, $p<0.001$). There was also an interaction effect between exercise and sex on nicotine consumption in mice given access to the single nicotine concentration paradigm ($F=16.61$, $p<0.001$). Female mice given access to the 100

ug/ml throughout the protocol showed a main effect of exercise on both nicotine consumption ($F=59.189$, $p<0.001$) and on nicotine preference ($F=55.97$, $p<0.001$) (Figures 1b and 2b). Female mice with access to a running wheel consumed (0.79 ± 0.9 mg/kg of nicotine per day) and preferred (0.03 ± 0.03) nicotine less than their sedentary counterparts (7.21 ± 4.44 mg/kg nicotine per day and 0.31 ± 0.18 respectively). Male mice given access to the single concentration nicotine paradigm did not show a significant effect of exercise on nicotine consumption, but they did show a main effect of exercise on nicotine preference ($F=10.18$, $p<0.01$) (Figures 1b and 2b). Male mice with access to a running wheel preferred nicotine less (0.14 ± 0.14) than their sedentary counterparts (0.24 ± 0.18).

Mice given access to a running wheel and graded nicotine concentration 50 μg/mL for four days followed by 100 μg/mL for 8 days (treatment 4) significantly consumed ($F=11.617$, $p<0.001$) and preferred ($F=23.26$, $p<0.001$) less nicotine than mice housed in an empty cage and graded nicotine concentrations (treatment 3) over the course of 12 days (Figures 1c and 2c). There was a main effect of sex on nicotine consumption for mice given access to the graded nicotine paradigm ($F=4.14$, $p<0.05$). There was also an interaction effect between exercise and sex on nicotine preference for mice with access to the graded nicotine paradigm ($F=7.26$, $p<0.01$). Female mice given access to the graded nicotine paradigm showed no significant effect of exercise on nicotine consumption or on nicotine preference (Figures 1a and 2a). Male mice given access to the graded nicotine concentration showed a main effect of exercise on both nicotine consumption ($F=30.1$, $p<0.001$) and on nicotine preference ($F=56.11$, $p<0.001$) (Figures 1a and 2a). Male mice with

access to a running wheel consumed (1.41 ± 1.45 mg/kg nicotine per day) and preferred (0.07 ± 0.06) less nicotine than their non-running counterparts (5.48 ± 3.3 mg/kg nicotine per day and 0.39 ± 0.21 respectively).

2.2 Mice

As expected, there was a significant difference ($F=308.15$, $p<0.001$) in body weight between female mice (21.97 ± 0.81) and male mice (26.19 ± 1.38). In female mice, mice with no running wheel and access to 100 ug/ml throughout the experiment had a significantly lower body weight (21.12 ± 0.48) than mice with a running wheel and access to 100 ug/ml throughout (22.15 ±0.49 g, $p<0.01$), mice with no running wheel and access to graded nicotine (22.03 ± 0.92 g, $p<0.01$), and mice with a running wheel and access to graded nicotine (22.59 ± 0.46 g, $p<0.001$). Male mice with a running wheel and graded nicotine had an average body weight greater (27.62 ± 1.67 g) than both mice with an empty cage and 100 ug/ml throughout (25.96 ± 1.07 g , $p<0.001$) and mice with a wheel and 100 ug/ml throughout (25.59 ± 0.87 g). Mice with no running wheel and access to graded nicotine had a greater body weight (26.91 ± 1.74 g) than mice with a running wheel and access to 100 ug/ml throughout (25.59 ± 0.87 g, $p<0.05$).

The distance ran increased over the 15 day protocol (Figure 3a**,** $F=3.12$, $p<0.01$) for both nicotine concentration paradigms. There was also a main effect of treatment (Figure 3a, $F=7.89$, $p<0.01$). There was a main effect of sex on time ran (Figure 4a, $F=0.04$, $p<0.05$), female mice ran 419.81 ± 66.5 minutes per day on average and male mice ran an average of 312.68 ± 102.86 minutes per day (Figure 4a). Females had an interaction effect between treatment and day on running time

(Figure 4a**,** F=3.19, p<0.001). Male mice showed a main effect of treatment on running time (Figure 4a, F=26.45, p<0.001). Running speed increased over the 15 day protocol (Figure 5a, F=13.13, p<0.001). There was also a main effect of sex on running speed (Figure 5a, F=6.54, p<0.05) whereby female mice ran an average speed of 1.37 ± 0.15 km/hr and male mice ran an average speed of 1.49 ± 0.26 km/hr. Female mice showed no significant differences in running speed. Male mice showed a main effect day on running speed (Figure 5a, F=7.67, p<0.001).

3. Discussion

3.1 Evidence for hedonic substitution

This study provides evidence for the behavioral interaction between voluntary exercise and nicotine consumption. Similar to previous studies from our lab involving exercise and ethanol consumption [17,18], we found that adult C57Bl/6J mice consumed and preferred less nicotine when given access to a running wheel compared to their non-running counterparts. The main study will assess whether or not this decrease in nicotine consumption and preference is directly due to exercise by having a third cage condition of a locked wheel [17].

3.2 Decision on which nicotine concentration to use

We decided to use the graded nicotine paradigm in which mice were given 50 μg/mL for the first four days followed by 8 days of 100 μg/mL. We decided to use this paradigm because there was a larger spread between the running and non-running groups (Figures 1c and 2c). Even though there was a different effect in each sex, we decided that by using the graded nicotine solution we would hopefully see

the larger difference between runners and non-runners in the main study that we observed in the pilot in both sexes combined.

Main Study

1. Methods

1.1 Behavioral paradigm

Mice were tested using a previously established paradigm that led to differences in ethanol consumption when given access to a free running wheel [17,18]. The six different conditions included:

1. Empty cage with access to water only (n=11) [5 female, 6 male]
2. Empty cage with access to one bottle water, one bottle nicotine two-bottle choice (n=11) [5 female, 6 male]
3. Locked wheel with access to water only (n=11) [5 female, 6 male]
4. Locked wheel with access to one bottle water, one bottle nicotine two-bottle choice (n=11) [5 female, 6 male]
5. Running wheel with access to water only (n=11) [5 female, 6 male]
6. Running wheel with access to one bottle water, one bottle nicotine two-bottle choice (n=10) [5 female, 5 male]

The protocol lasted 15 days, including a three-day acclimation period during which all mice were given access to water only. Mice housed with a running wheel had 24-hour access to the wheel for all 15 days. Running distance, speed and time were recorded using a magnet and a bike speedometer. The side of the cage the bottles were on was switched every two days as to minimize placement preference.

Individual water and nicotine levels (if applicable) were recorded daily. Mice housed with nicotine two-bottle choice were given 50 μg/ml nicotine days 1-4 followed by 100 μg/ml nicotine days 5-12. On day 12 of nicotine treatment mice were sacrificed by cervical dislocation. Blood samples were collected by cardiac puncture, full brains were taken, and liver weights were recorded.

1.1 Statistical Analysis

All statistics were done using RStudio version 0.98.501 (www.rstudio.com). Measurements from days immediately following the weighing of mice (experimental days 1, 5, and 9) were excluded to control to behavioral differences that could be caused by animal handling. Due to high variability in nicotine consumption and preference between days, and average of days 6-12 was used for each animal. A two-way ANOVA (Sex x Cage Condition) was used to assess any differences in nicotine consumption and preference. Within each sex, a one-way ANOVA was used to determine differences between the three cage conditions (empty cage, locked wheel, running wheel). Two-way repeated measure ANOVAs were used to determine differences in runners' speed, distance and time (males vs. females and nicotine drinkers vs. water only non-drinkers). Within each sex one-way repeated measures ANOVAs were used to determine differences running speed, distance, and time (nicotine drinkers vs. water only non-drinkers). Two-way ANOVAs were used to look at sex differences in body weight, food consumption, and liver weight (Sex x Treatment Condition). Within each sex a one-way ANOVA was used to look at any differences in treatment. Any missing values (due to spontaneous bottle or counter

malfunction) were imputed by averaging the day before and the day after the missing data.

2. Results

2.1 Voluntary running and nicotine consumption

There was no significant effect of cage condition on nicotine consumption or preference in either male or female mice.

There was a significant effect of sex on total volume of liquid consumption ($F=29.53$, $p<0.001$). Female showed a main effect of day ($F=2.3$, $p<0.5$) indicating that total volume intake increased slightly in the first few days of the experiment. Male mice showed a main effect of treatment on total volume consumed ($F=18.05$, $p<0.001$) whereby mice given access to a wheel drank a significantly higher total volume than mice with an empty cage or mice with a locked wheel regardless of access to nicotine.

2.2 Mice

Body weights did not significantly change over the course of the experiment. As expected, females weighed significantly less (20.58 g ± 1.47 g) than males (25.74 g ± 2.23 g) ($F=355.94$, $p<0.001$). In females there was a main effect of treatment group on body weight (Table 1, $F=34.46$, $p<0.001$). There was no significant difference in body weight for male mice between treatment groups (Table1). There was a main effect of treatment on food consumption (Figure 6, $F=10.89$, $p<0.001$). Mice given access to a running wheel ate more food than both mice in empty cages and mice in cages with a locked wheel. The difference in food consumption between

runners and non-runners is most likely due to an increased caloric output and the reciprocal need for greater caloric input. There was no effect of sex on food consumption.

There was an increase in distance run in both sexes over the 15 day protocol (Figure 3b**,** $F=20.87$, $p<0.001$). There was main effect of sex on the distance run ($F=13.77$, $p<0.001$). Female mice with access to a running wheel ran significantly farther (11.27 ± 2.28 km/day) than male mice with access to a running wheel (9.79 ± 2.28 km/day). There was no effect of access to nicotine on distance run in either female or male mice (Figure 3b). Female runners also ran significantly longer (455.32 ± 57.51 min) than male runners (365.14 ± 56.83 min; $F=121.05$, $p<0.001$) (Figure 4b). There was no effect of access to nicotine on time spent running in females or males (Figure 4b). The speed at which mice ran increased throughout the 15 day protocol (Figure 5b, $F=40.875$, $p<0.001$). Male mice ran faster on average (1.58 ± 0.23 km/hr) than female mice (1.46 ± 0.19 km/hr; $F=66.818$, $p<0.001$)(Figure 5b). There was no effect of nicotine on speed in female mice (Figure 5b). There was an interaction effect between access to nicotine and day on running speed in male mice (Figure 5b, $F=19.3$, $p<0.01$).

2.3 Liver weights

Livers were standardized for body weight. There initially was a main effect of treatment on liver weight ($F=2.86$, $p<0.05$). However, after doing a pairwise t-test and using a bonferroni correction for multiple testing, there was no longer any significant differences between groups (Figure 7).

3. Discussion

3.1 Nicotine affects males and females differently

These data further support the existing evidence that nicotine affects males and females differently. While not significant, female mice consumed slightly more nicotine than males (7.53 ± 4.12 mg/kg vs. 5.70 ± 2.89 mg/kg respectively). This trend is consistent with existing data [30,31] that females consume more nicotine than males. While there were no significant effects of cage condition on nicotine consumption or preference, there was a trend in the female data that mice with access to a running wheel consumed and preferred less nicotine than mice with and empty cage and mice with a locked wheel (Figures 1d and 2d). This trend follows similar findings in previous studies from our lab involving exercise and alcohol consumption [17,18].

These results are contradictory to the pilot study data, which suggested that access to a running wheel decreased nicotine consumption and gave evidence of hedonic substitution. This discrepancy may be largely due to the small sample size at this point in the study. Due to such a small population size, these preliminary results do not hold much statistical power and further research is required.

4. Conclusions

As the data were not consistent between the pilot study and main study, a larger sample size is needed to further assess the effect of exercise on nicotine consumption in adult C57BL/6J mice. These findings did show a difference in sex with regard to exercise and nicotine behaviors. Our lab plans to further this research by increasing the sample size of the study. Additionally, the plasma

samples taken from the mice will be analyzed for the nicotine metabolite cotinine in order to assess differences in metabolism with a larger sample. Brain tissue may be analyzed in the future to determine changes in gene expression in certain regions previously found to be associated with the mesolimbic reward pathway.

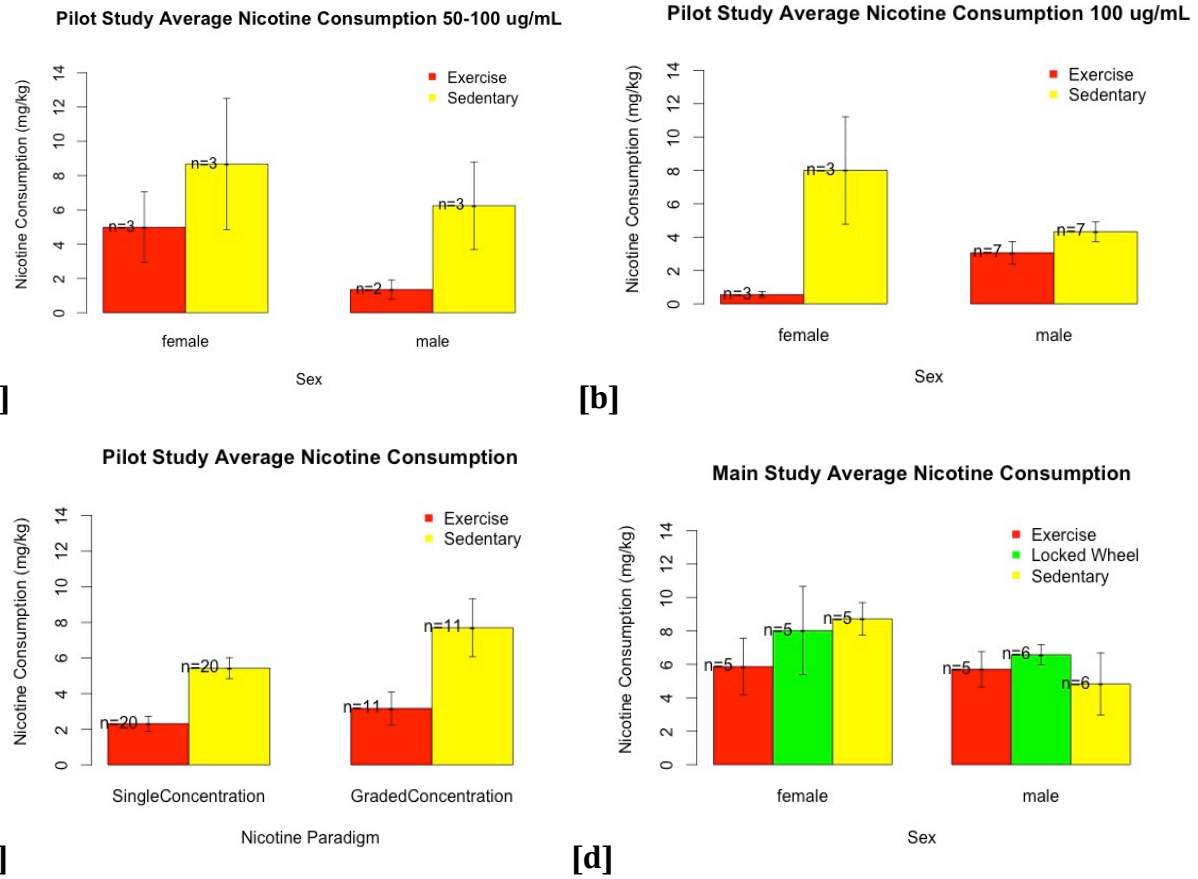


Figure 1: Nicotine Consumption. [a] Pilot data – effect of exercise on average nicotine consumption for mice given access to the graded nicotine concentration paradigm for both males and females. **[b]** Pilot data – effect of exercise on average nicotine consumption for mice given access to the single nicotine concentration paradigm for both males and females. **[c]** Pilot data – effect of exercise on average nicotine consumption comparing the two concentration paradigms. **[d]** Main data – effect of cage condition on average nicotine consumption for males and females. *(All data presented as mean of experimental days 6-12 ± Standard Error of the mean)

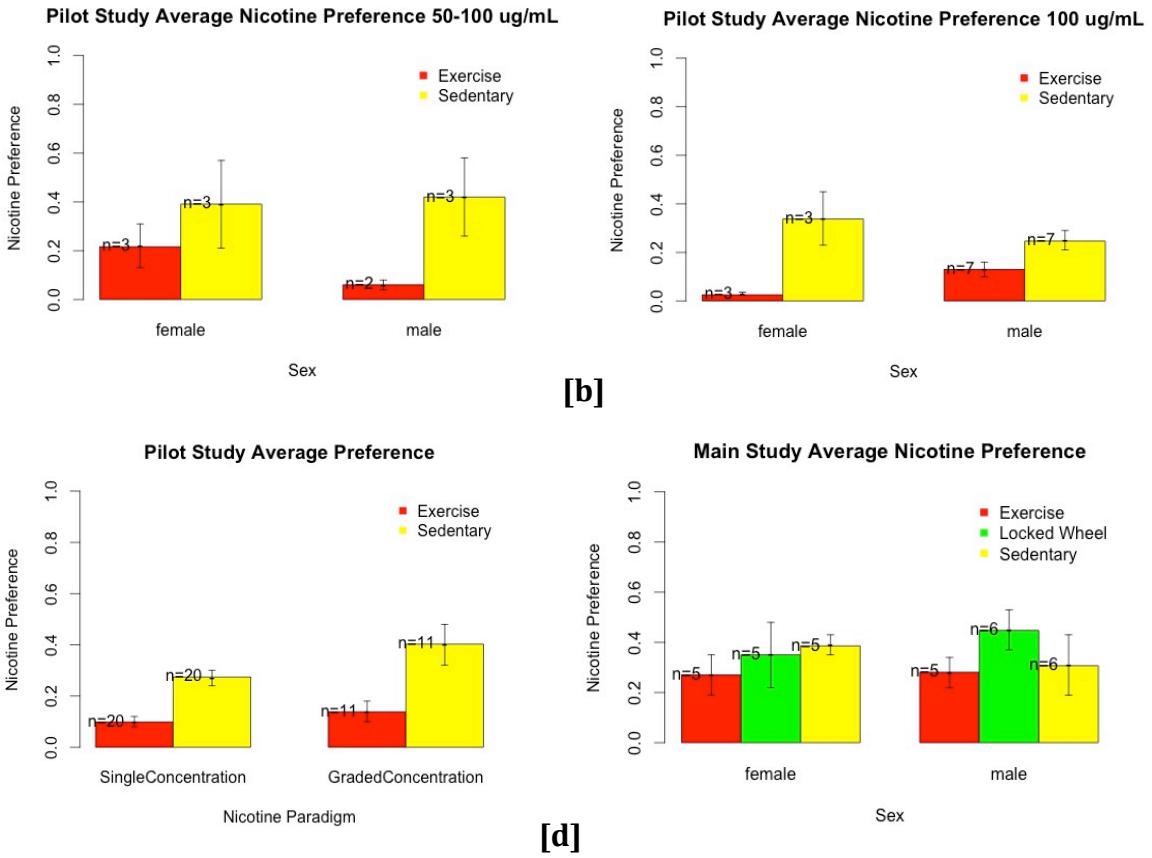
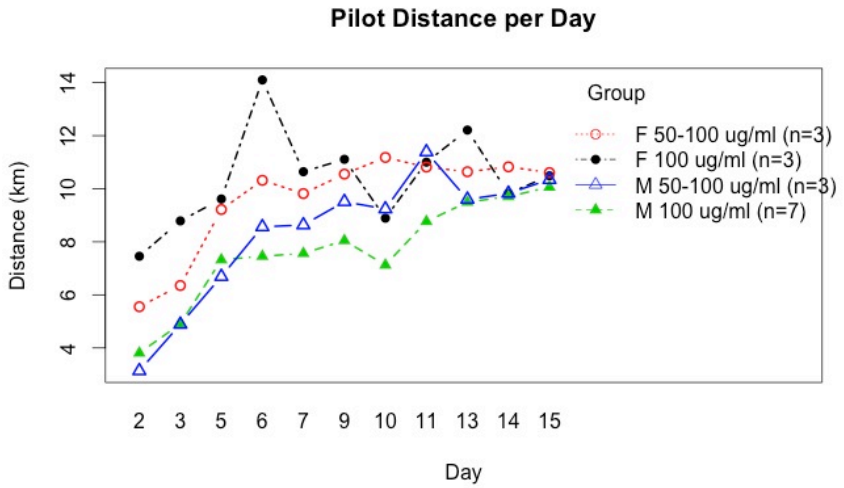


Figure2: Nicotine Preference. [a] Pilot data – effect of exercise on average nicotine preference for mice given access to the graded nicotine concentration paradigm for both males and females. **[b]** Pilot data – effect of exercise on average nicotine preference for mice given access to the single nicotine concentration paradigm for both males and females. **[c]** Pilot data – effect of exercise on average nicotine preference comparing the two concentration paradigms. **[d]** Main data – effect of cage condition on average nicotine preference for males and females. *(All data presented as mean of experimental days 6-12 ± Standard Error of the mean)



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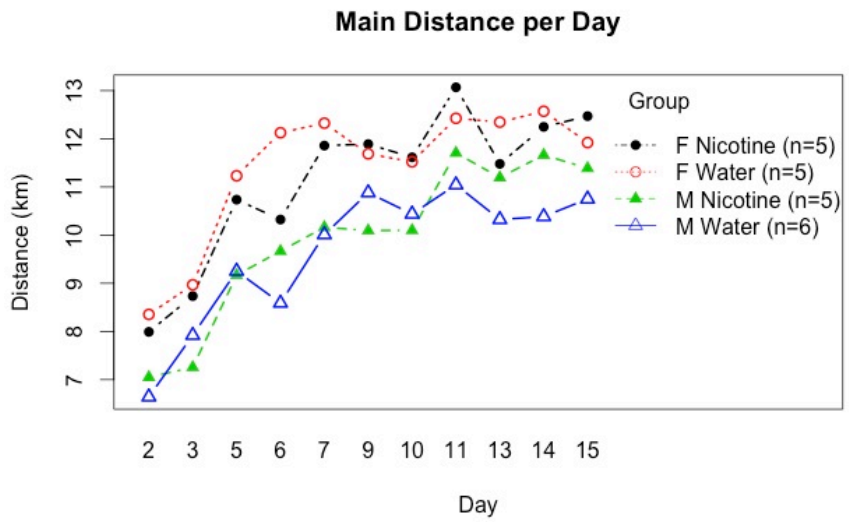
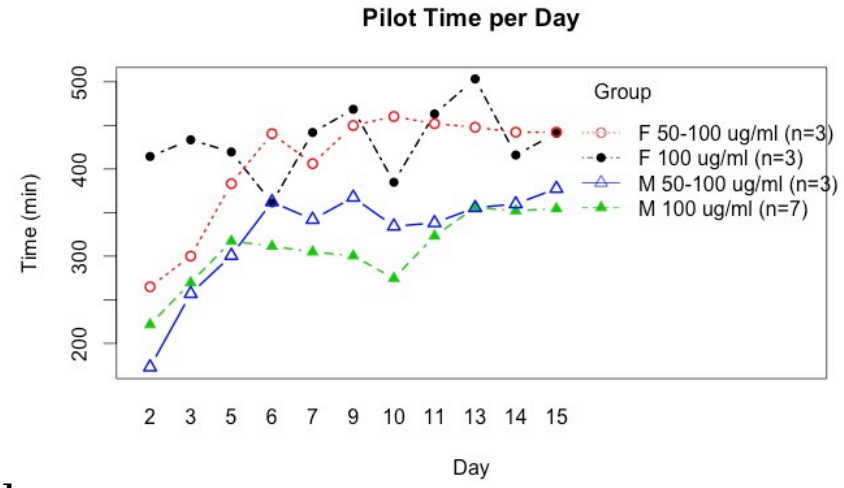


[b]

Figure 3: Running Distance. [a] Pilot study exercise data comparing sexes and nicotine paradigm distance run (km) per day. **[b]** Main study exercise data comparing sexes and access to nicotine distance run (km).



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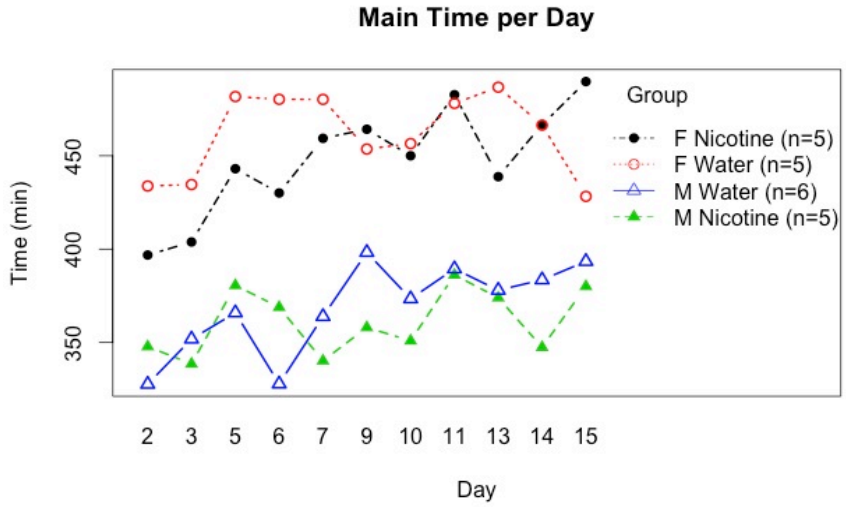
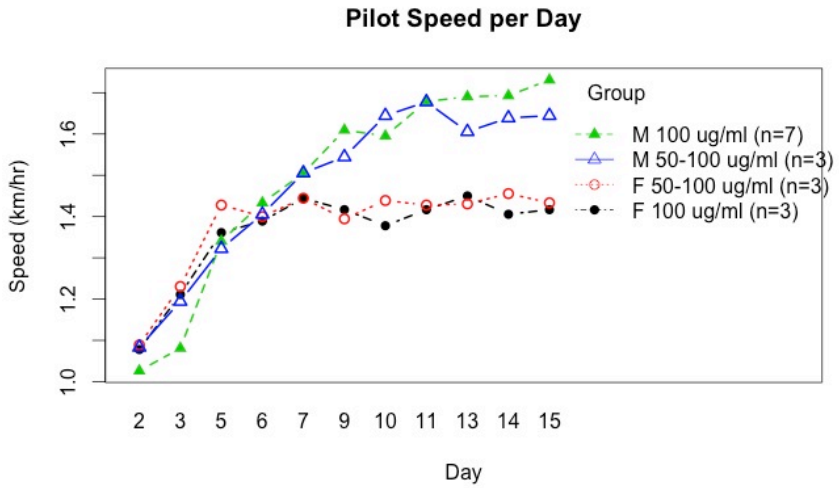


[b]

Figure 4: Running Time. [a] Pilot study exercise data comparing sexes and nicotine paradigms time run (min). **[b]** Main study exercise data comparing sexes and access to nicotine time run (min).



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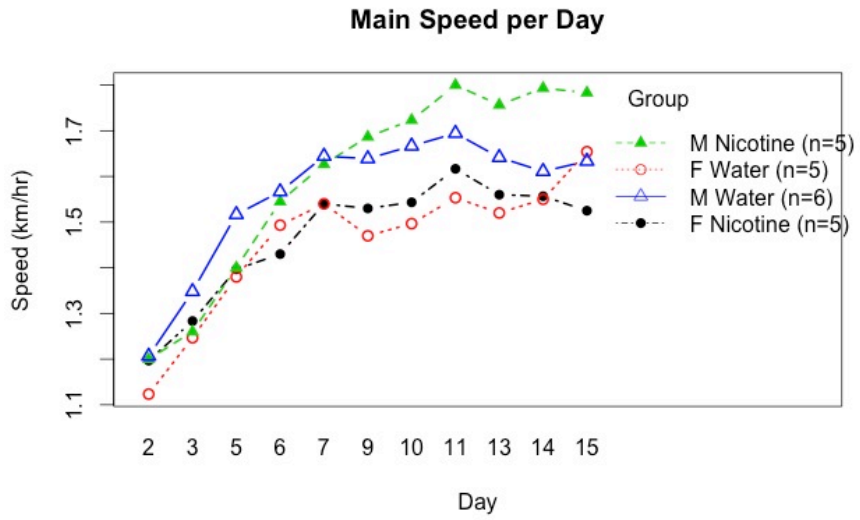

[b]

Figure 5: Running Speed. [a] Pilot study exercise data comparing sexes and nicotine paradigm running speed (km/hr). **[b]** Main Study exercise data comparing sexes and access to nicotine running speed (km/hr).

Sex	Empty Cage + Water Only	Empty Cage + Two-Bottle Choice	Locked Wheel + Water Only	Locked Wheel + Two-Bottle Choice	Running Wheel + Water Only	Running Wheel + Two-Bottle Choice
Female	19.88 ± 0.98 g	20.08 ± 1.26 g	19.88 ± 1.29 g	21.05 ± 1.06 g	21.46 ± 1.95 g	21.22 ± 1.02 g
Male	25.17 ± 1.48 g	25.82 ± 2.20 g	26.05 ± 2.40 g	25.64 ± 2.70 g	24.86 ± 1.55 g	24.27 ± 1.15

Table 1: Body Weight. Average body weight for animals in each treatment group. (Data presented as mean ± standard deviation)

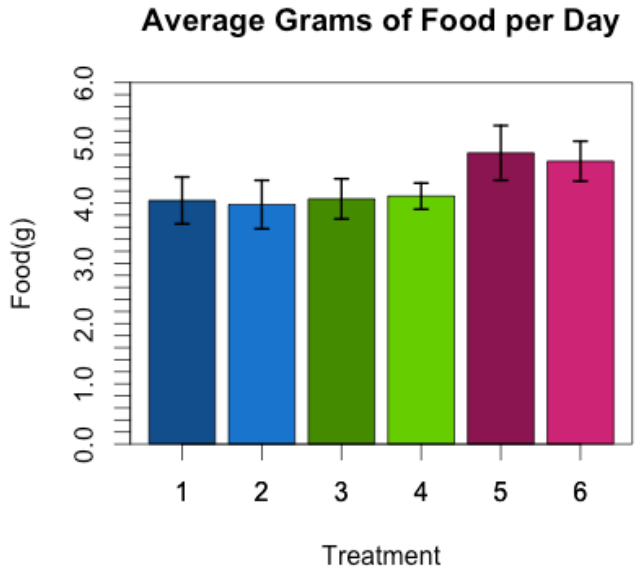

Figure 6: Food per Day. Average grams of food per day comparing treatment groups. *(Data presented as mean ± Standard Error of the mean)

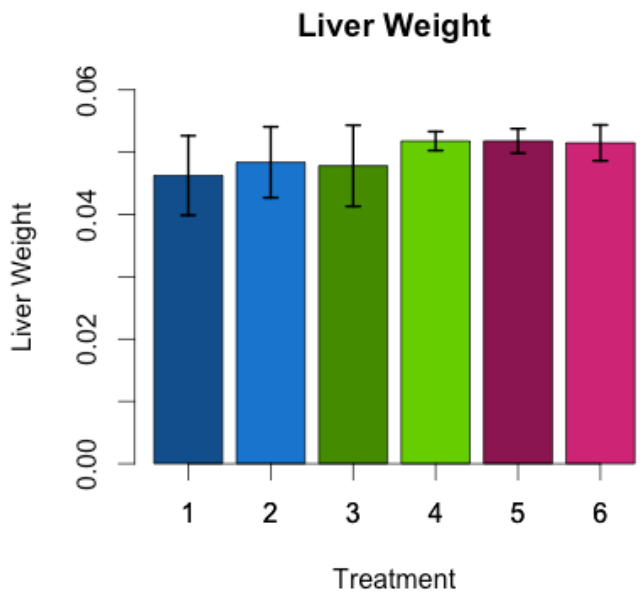

Figure 7: Liver Weights. Comparing liver weight adjusted for body weight between treatment groups. *(Data presented as mean ± Standard Error of the mean)

References:

1. CDC | Centers for Disease Control and Prevention

2. American Society of Addiction Medicine. **Public Policy Statement on Nicotine Dependence and Tobacco**. Chevy Chase (MD): American Society of Addiction Medicine, 2010 [accessed 2014 Feb 7].

3. Lopez-Quintero, C., et al. Probability and predictors of transition from first use to dependence on nicotine, alcohol, cannabis, and cocaine: Results of the national epidemiologic survey on alcohol and related conditions (nesarc). Drug Alcohol Depend 2010, 115(1-2): 120-130.

4. Ball, D. Addiction science and its genetics. Addiction 2008, 103(3): 360-367.

5. Schlaepfer IR, Hoft NR, Ehringer MA. The genetic components of alcohol and nicotine co-addiction. Current Drug Abuse Reviews 2008, 1(2): 124-134.

6. Albuquerque EX, Pereira EFR, Alkondon M, et al. Mammalian Nicotinic Acetylcholine Receptors: From Structure to Function. Physiol Rev 2009, 89(1): 73-120.

7. Dierker LC, Rose JS, Donny E, et al. Alcohol Use as a Signal for Sensitivity to Nicotine Dependence Among Recent Onset Smokers. Addict Behav 2011, 36(4): 421-426.

8. Hendrickson LM, Guildford MJ, and Tapper AR. Neuronal Nicotinic Acetylcholine Receptors: Common Molecular Substrates of Nicotine and Alcohol Dependence. Front Psychaitry 2013, 4(29).

9. Willuhn I, Wanat MJ, Clark JJ, et al. Dopamine Signaling in the Nucleus Accumbens of Animals self-administration of Drugs of Abuse. Curr Top Behav Neurosci 2010, 3: 29-71. [PubMed: 3766749]

10. Tizabi Y, Bai L, Copeland RL, Taylor RE, et al. Combined effects of systemic alcohol and nicotine on dopamine release in the nucleus accumbens shell. Alcohol and Alcoholism, 2007, 42(5): 413-416.

11. Ding ZM, Katner SN, Rodd ZA, et al. Repeated exposure of the posterior ventral tegmental area to nicotine increases the sensitivity of local dopamine neurons to the stimulating effects of alcohol. Alcohol 2012, 46(3): 217-223.

12. McMillan DE. Effects of access to a running wheel on ethanol intake in rats under schedule-induced polydipsia. Curr Alcohol 1978;3:221–35.

13. McMillan DE, McClure GY, Hardwick WC. Effects of access to a running wheel on food, water and ethanol intake in rats bred to accept ethanol. Drug Alcohol Depend 1995;40:1–7.

14. Werme M, Lindholm S, Thorén P, Franck J, Brené S. Running increases ethanol preference. Behav Brain Res 2002;133:301–8.

15. Ozburn AR, Harris RA, Blednov Y a. Wheel running, voluntary ethanol consumption, and hedonic substitution. Alcohol 2008;42:417–24.

16. Hammer SB, Ruby CL, Brager AJ, Prosser RA, Glass JD. Environmental modulation of alcohol intake in hamsters: effects of wheel running and constant light exposure. Alcohol Clin Exp Res 2010;34:1651–8.

17. Ehringer MA, Hoft NR, Zunhammer M. Reduced alcohol consumption in mice with access to a running wheel. Alcohol 2009;43:443–52.

18. Darlington TM, McCarthy RD, Cox RJ, et al. Mesolimbic transcriptional response to hedonic substitution of voluntary exercise and ethanol consumption. Behav Brain Res 2014; 259: 313-320.

19. Kalivas PW, Volkow ND. The Neural Basis of Addiction : A Pathology of Motivation and Choice. Am J Psychaitry 2005; 162: 1403-1413.

20. Leasure JL, Nixon K. Exercise Neuroprotection in a Rat Model of Binge Alcohol Consumption. Alcohol Clin Exp Res 2010; 34(3): 404-414.

21. Zlebnik NE, Anker JJ, Carroll, ME, et al. Reduction of extinction and reinstatement of cocaine seeking by wheel running in female rats. Psychopharmacology (Berl) 2010; 209(1): 113-125.

22. Willuhn I, Wanat, MJ, Phillips, PEM, et al. Dopamine Signaling in the Nucleus Accumbens of Animals Self-Administering Drugs of Abuse. Curr Top Behav Neurosci 2010; 3: 29-71.

23. Biasi, MD and Dani, JA. Reward, Addiction, Withdrawal to Nicotine. Annu Rev Neurosci 2011; 34:105-130.

24. Sanchez V, Catherine MF, Brunzell DH, et al. Sex differences in the effect of wheel running on subsequent nicotine-seeking in a rat adolescent-onset self-administration model. Psychopharmacology (Berl) 2013.

25. Butt CM, Hutton SR, Stizel JA, et al. A polymorphism in the alpha4 Nicotinic Receptor Gene (Chrna4) Modulates Enhancement of Nicotine Receptor Function by Ethanol. Alcohol Clin Exp Res 2004; 27(5): 733-742)

26. Marszalec W, Aistrup GL, Huang CS, et al. Ethanol-nicotine interactions at alpha-bungarotoxin-intensive nicotinic acetylcholine receptors in rat cortical neurons. Alcohol Clin Exp Res 1999; 23(3): 439-449.

27. Gould TJ, Collins AC, Wehner JM. Nicotine enhances latent inhibition and ameliorates ethanol-induced deficits in latent inhibition. Nicotine Tob Res 2001; 3(1): 17-24.

28. Tritto T, Marley RJ, Bastidas, D et al. Potential regulation of nicotine and ethanol actions by alpha 4-containing nicotinic receptors. Alcohol 2001; 24(2): 171-5.

29. Butt CM, King NM, Stitzel JA, et al. Interaction of the nicotinic cholinergic system with ethanol withdrawal. J Pharmacol Exp Ther 2004; 308(2): 591-599

30. Klein LC, Stine MM, Vandenbergh DJ, et al. Sex Differences in voluntary oral nicotine consumption by adolescent mice: a dose-response experiment. Pharmacology Biochemistry and Behavior 2004; 78(1): 13-25.

31. Roth ME, Cosgrove KP, and Carroll ME. Sex differences in the vulnerability to drug abuse: a review of preclinical studies. Neuroscience & Biobehavioral Reviews 2004; 28(6): 533-546

32. Benwell MEM, Balfour DJK, and Anderson JM. Evidence that tobacco smoking increases the density of (-)-[3H]nicotine binding sites in human brain. J Neurochem 1988; 50(4): 1243-1247.

33. True WR, Xian H, Scherrer JF, et al. Common genetic vulnerability for nicotine and alcohol dependence in men. Arch Gen Psychiatry 1999; 56(7): (655-661).