UNIVERSITY OF CALIFORNIA,
IRVINE

Drug Addiction - the Research & the Researchers:

The Lasting Impact of Adolescent Nicotine and Cannabinoid Exposure
and
The Importance of Effectively Supporting Historically Marginalized Scientists

DISSERTATION

submitted in partial satisfaction of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

in Biological Sciences

by

Angeline Joan Dukes

Dissertation Committee:
Associate Professor Christie Fowler, Chair
Professor Marcelo Wood
Associate Professor Stephen Mahler

2022

DEDICATION

To my family for their unwavering support
especially my dad, Coulange Eugene, my Grandma Joan,
and my aunties Sandy, Dottie and Indira.
Although you may not have fully understood what I was doing in lab for the past 5 years,
I appreciate you believing in me and showing up when I needed you.

To my husband, Jovon Dukes,
without whom this would not have been possible.
From waiting in the parking lot when my experiments took longer than I expected, to attending every presentation and celebration, to talking me down and hyping me up when I wanted to give up. Thank you for always reminding me that this PhD journey is a part of me but doesn't define me.
I love you.

My Black In Neuro family,
You are the hope for the future.
This community is perhaps my greatest achievement, and I am so grateful to have you.

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ACKNOWLEDGEMENTS

I will forever be grateful to my committee chair, Professor Christie Fowler, for her continual guidance and support during this journey. From her patience as I held a mouse for the first time to her understanding as I navigated the intersections of my identities as a scientist and a Black woman, I deeply appreciate having her as my mentor. Dr. Fowler has helped mold me into a confident researcher who is unafraid of giving her opinions or voicing her concerns. She has supported me as I found a bridge between the science that we do and the impact I want to leave in this world. She has encouraged all of my outreach, diversity and inclusion work, and reinforced that it is truly valued. I look forward to the collaborations we will undoubtedly have in the future to make neuroscience more equitable and accessible.

I would also like to thank my committee members, Professor Marcelo Wood and Professor Steve Mahler. They have both been overwhelmingly encouraging and offered critical guidance in so many ways. They have supported my career aspirations and goals both in and outside of the lab. Together as a committee with Dr. Fowler, they helped me find a balance between my novel research, my love for teaching, and my important DEI work. I am grateful that they value all the ways I hope to make a difference in academia.

I would like to thank all past and current members of the Fowler lab. Valeria has been the most wonderful postdoc mentor I could have ever hoped for; from her patience through my confusion to her reassurance that I can do this work, she would always take the time to help me, even when she was super busy herself. My fellow graduate students, Yasmine, Andrew, Malia, and Sam have been incredible sources of support as we struggled through learning surgeries, figuring out run schedules, and offering encouragement as we wrote papers, practiced presentations, and cried from life's problems. Thank you all for being such good friends. All of the lab techs and undergrads in the lab through the years, especially Edison, Amina, Anna, and Adriana, thank you for your help through all of these endless experiments, for the laughs, and the memories.

To my INP cohort, Pedagogical Fellows cohort, and UCI family, thank you for always showing up, offering laughs, and reminders that a good game night or bonfire can recharge you after any rough day. Especially to my grad school bestie, Elena Dominguez who has been there through all the hard times. Thank you, Elena, for always being an ear to empathize with and co-conspirator in the fight to make neuroscience better for those coming after us. This journey would have been so much harder without you.

To my godchildren, Fisk family, and best friends, thank you for reminding me of my life outside of lab and being there when I needed to take breaks. Thank you for emphasizing that I CAN do this, especially when I wanted to give up. Fisk gave me the support system that I needed to embark on this journey and the courage to finish it. Thank you to my former professors Lee Limbird, Patricia McCarroll, Phyllis Freeman, and Natalie Arnett for showing me that this career path is an option and believing in me when I didn't believe in myself. I hope to give back to current and future Fiskites in the same way, because science is for everyone.

To my external mentors and Black In Neuro family, Monica Gaudier-Diaz, Nii Addy, Melissa Herman, Mike Yassa, Manuella Yassa, Theanne Griffith, Marguerite Matthews, and all of the Black In Neuro organizing team, THANK YOU for your guidance in this journey. Thank you for listening to my problems and helping me find the best solutions. Thank you for reminding me that it's ok to put my goals and dreams first and to fight for them no matter what anyone else wants me to do. Thank you for taking the time out of your busy schedules to teach me how to be a better mentee, mentor, teacher, scientist, and person. Thank you for advocating for me.

I want to thank the funding sources that made this research possible and everyone who helped me successfully attain the funding. The research in this dissertation was supported by my National Science Foundation Graduate Research Fellowship, which I successfully attained in my first year of graduate school with guidance from Christie Fowler, Fiona Harrison, Mike Mulligan, and the Graduate Division at UC Irvine. This research was also supported through the National Institute of Drug Abuse T32 Predoctoral Training Grant, directed by Marcelo Wood. Thank you to the NIH, NSF, and NIDA for funding this important work.

Furthermore, an enormous thank you to all of my co-authors on the published works within this dissertation. Thank you to my Fowler lab colleagues, Malia Bautista, Valeria Lallai, Anna Pushkin, Yasmine Sherafat, Adriana Hernandez Vasquez, James Fowler, Yen-Chu Chen, Edison Chen, Alan Torres-Mendoza, and of course, Dr. Christie Fowler for their work and support in publishing "Impact of Δ^9-tetrahydrocannabinol (THC) and Synthetic "Spice" Cannabinoids on Nicotine Use and Abuse" (Cannabis Use, Neurobiology, Psychology, and Treatment), "Cannabinoid and Nicotine Exposure during Adolescence Induces Sex-Specific Effects on Anxiety- and Reward-Related Behaviors during Adulthood" (Plos One), and "Adolescent Cannabinoid and Nicotine Exposure Alters Nicotine Self-Administration in Adulthood" (Nicotine & Tobacco Research). I would also like to thank my Black In Neuro co-organizers, Kaela Singleton, Rackeb Tesfaye, Elena Dominguez, De-Shaine Murray, and Lietsel Richardson for their wonderful contributions in publishing "An Open Letter to Past, Current, and Future Mentors of Black Neuroscientists" (Nature Reviews Neuroscience), "A Year in Review: Are Diversity, Equity, and Inclusion Initiatives Fixing Systemic Barriers?" (Neuron), and "How to Better Support Black Trainees in the Biomedical Sciences" (Nature Medicine). These publications are included with their permission.

Finally, I want to thank all of the administrative staff, animal research care technicians, and custodians who allow the research to run smoothly. Especially to the administrative team in the Department of Neurobiology & Behavior and the CNLM, thank you Naima, Maggie, Sally, and Diana. The work you do it crucial to our success as scientists and my ability to earn this degree. You are often unsung heroes, but from every conversation I have had with you to the encouragement you give me in passing, I appreciate you.

VITA
Angeline J. Dukes

Education

Ph.D.	Biological Sciences, concentration in Neuroscience University of California, Irvine	2022
M.Sc.	Biological Sciences, concentration in Neuroscience University of California, Irvine	2021
B.A.	Biology Fisk University, *Summa Cum Laude*	2017

Fellowships, Honors, and Awards

UCI Outstanding Graduate Student - Lauds & Laurels Award 2022
Alumni Association of the University of California, Irvine

Neuroscience Next 'One to Watch' Award 2021
Alzheimer's Association International Conference

Outstanding DECADE Representative Award 2021
University of California, Irvine – Graduate Division

2021 Commencement Speaker 2021
University of California, Irvine – School of Biological Sciences

Edward Steinhaus Teaching Award 2021
University of California, Irvine – School of Biological Sciences

Substance Use and Use Disorders T32 Pre-Doctoral Training Program 2021
National Institute on Drug Abuse, Grant No. DA050558-02

Most Promising Future Faculty Award 2021
University of California, Irvine – Division of Teaching, Excellence and Innovation

Summer Inclusive Excellence Grant 2021
University of California, Irvine – Graduate Division

Pedagogical Fellowship 2021
University of California, Irvine – Division of Teaching, Excellence and Innovation

2021 Travel Award for the Annual Society Meeting 2020
Society for Research on Nicotine and Tobacco

Exceptional Service Award for Diversity, Equity, and Inclusion Work 2020
University of California, Irvine – Department of Neurobiology and Behavior

Neuroscience Scholars Program Fellow 2020
Society for Neuroscience

2020 Health Disparities Network Travel Scholarship 2019
Society for Research on Nicotine and Tobacco

Best Datablitz Presentation Award 2019
Irvine Center for Addiction Neuroscience

Society for Neuroscience Travel Scholarship 2019
Summer Program in Neuroscience, Excellence, and Success

Norman A. Weinberger Award 2019
University of California, Irvine – Center for Neurobiology of Learning & Memory

National Science Foundation Graduate Research Fellowship (NSF GRFP) 2018
National Science Foundation, Grant No. DGE-1839285

2019 North American Cannabis Summit Registration Scholarship 2018
North American Cannabis Summit

Provost PhD Fellowship 2017
University of California, Irvine – Graduate Division

Diversity Recruitment Fellowship 2017
University of California, Irvine – Graduate Division

Publications

1. **Dukes AJ**, Lallai V, Hernandez Vasquez A, Bautista MR, Chen Y, Fowler JP, Sherafat Y, and Fowler CD. Edibles, Vaping, and Relapse: Examining the Lasting Impact of Adolescent Exposure to THC and/or Nicotine on Incubation of Nicotine Craving. *In Prep.*

2. **Dukes AJ**, Bautista MR, and Fowler CD. (2022) Impact of Δ^9-tetrahydrocannabinol (THC) and Synthetic "Spice" Cannabinoids on Nicotine Use and Abuse. *Cannabis Use, Neurobiology, Psychology, and Treatment.* Accepted.

3. Singleton KS, Murray DRK, **Dukes AJ**, and Richardson LNS. (2021) A year in review: Are diversity, equity, and inclusion initiatives fixing systemic barriers? Neuron. DOI: https://doi.org/10.1016/j.neuron.2021.07.014

4. Elayouby K, Ishikawa M, **Dukes AJ**, Smith A, Lu Q, Fowler CD, and Kenny PJ. (2020) Alpha3* Nicotinic Receptors in the Habenular-Interpeduncular Avoidance System Regulate Nicotine Intake. Journal of Neuroscience. DOI: 10.1523/JNEUROSCI.0127-19.2020

5. Singleton KS, Tesfaye R, Dominguez EN, and **Dukes AJ**. (2020) An Open Letter to Past, Current, and Future Mentors of Black Neuroscientists. Nature Reviews Neuroscience. DOI: 10.1038/s41583-020-00421-9

6. **Dukes AJ**. How to Better Support Black Trainees in the Biomedical Sciences. (2020) Nature Medicine. DOI: 10.1038/s41591-020-1101-3

7. **Dukes AJ**, Fowler JP, Lallai V, Pushkin AN, and Fowler CD. (2020) Adolescent Cannabinoid and Nicotine Exposure Alters Nicotine Self-Administration in Adulthood. Nicotine & Tobacco Research. DOI: 10.1093/ntr/ntaa084

8. Pushkin AN*, **Eugene (Dukes) AJ***, Torres-Mendoza A, Lallai V, Chen E, and Fowler CD. (2019) Cannabinoid and nicotine exposure during adolescence induces sex-specific effects on anxiety- and reward-related behaviors during adulthood. Plos One. DOI: 10.1371/journal.pone. 0211346 *co-first authors

9. Mi DJ, Dixit S, Warner TA, Kennard JA, Scharf DA, Kessler ES, Moore LM, Consoli DC, Brown CW, **Eugene (Dukes) AJ**, Kang JQ, and Harrison FE. (2018) Altered glutamate clearance in ascorbate deficient mice increases seizure susceptibility and contributes to cognitive impairment in APP/PSEN1 mice. Neurobiol Aging. *DOI:* 10.1016/j.neurobiolaging.2018.08.002

Presentations

Oral

1. **Dukes AJ**, Hernandez-Vasquez A, Bautista M, Chen Y and Fowler CD (2021) The Lasting Impact of Adolescent Cannabinoid Exposure on Adulthood Drug Seeking Behaviors in Mice. Cannabinoid GRS 2021 Meeting Planner. Venture, CA: Cannabinoid Function in the CNS Gordon Research Seminar.
2. **Dukes AJ** (2021) Undervalued Work and Unsung Heroes. OHBM 2021 Abstract Book. Virtual Conference: 2021 Organization for Human Brain Mapping Annual Meeting.

3. **Dukes AJ** and Fowler CD (2021) Adolescent Nicotine and THC Exposure Alters Cue-Induced Relapse in Adult Mice. Memory: It's About Time - Virtual CNLM Spring Conference 2021. Irvine, CA: Center for the Neurobiology of Learning and Memory.
4. **Dukes AJ**, Hernandez-Vasquez A, Sherafat Y, Bautista M, and Fowler CD (2021) Adolescent Nicotine and Cannabinoid Exposure Alters Susceptibility to Cue-Induced Nicotine Relapse Later in Life. SRNT 2021 Abstract Book/Meeting Planner. Virtual Conference: Society for Research on Nicotine and Tobacco Annual Meeting.
5. Sherafat Y, Bautista M, Lallai V, **Dukes AJ**, Fowler JP, and Fowler CD (2021) The Nicotinic Receptor Modulator Lynx1 in Nicotine Reinforcement. SRNT 2021 Abstract Book/Meeting Planner. Virtual Conference: Society for Research on Nicotine and Tobacco Annual Meeting.
6. Savage LM, **Dukes AJ**, Khokhar JY, and Ramirez S (2021) Diversity Builds a Better Neuroscience. SfN Global Connectome Virtual Event Guide. Virtual Conference: Society for Neuroscience.
7. **Dukes AJ**, Pushkin A, Lallai V, Fowler JP, Hernandez-Vasquez A, Sherafat Y, and Fowler CD (2020) Adult Nicotine Self-Administration and Relapse-Related Behavioral Effects Following Adolescent Exposure to Nicotine and a Cannabinoid Agonist. SRNT 2020 Abstract Book/Meeting Planner. New Orleans, LA: Society for Research on Nicotine and Tobacco Annual Meeting.
8. Fowler CD and **Dukes AJ** (2020) The Nicotinic Receptor Modulator, Lynx2, Influences Nicotine Intake and Relapse-related Behaviors. SRNT 2020 Abstract Book/Meeting Planner. New Orleans, LA: Society for Research on Nicotine and Tobacco Annual Meeting.
9. **Dukes AJ** and Fowler CD (2019) Sex-Specific Effects in Adulthood Nicotine Self-Administration and Incubation of Craving Following Adolescent Nicotine and Cannabinoid Exposure. Annual ICAN Symposium. Irvine, CA: Irvine Center for Addiction Neuroscience.
10. **Dukes AJ** and Fowler CD (2019) Differential Incubation of Nicotine Craving Effects in Male and Female Mice Following Adolescent Exposure to Nicotine and a Cannabinoid Agonist. Annual SPINES Symposium. Woods Hole, MA: Summer Program in Neuroscience, Excellence, and Success.
11. **Eugene (Dukes) AJ** and Fowler CD (2019) Altered Relapse-Related Behaviors Following Adolescent Exposure to Nicotine and a Cannabinoid Agonist in Adult Mice. CNLM 2019 Annual Meeting. Irvine, CA: Center for the Neurobiology of Learning and Memory.
12. **Eugene (Dukes) AJ**, and Fowler CD (2017) The Effects of Adolescent Nicotine and Cannabinoid Exposure on Nicotine Self-Administration during Adulthood. 2017 Competitive Edge Research Symposium. Irvine, CA: University of California, Irvine Graduate Division.

Poster

13. **Dukes AJ**, Hernandez-Vasquez A, Sherafat Y, Bautista M, and Fowler CD (2020) Does Exposure to Nicotine and Cannabinoids during Adolescence Make Nicotine Relapse More Likely in Adulthood? 59th ACNP Annual Meeting Planner. Virtual Conference: American College of Neuropsychopharmacology.
14. **Dukes AJ**, Pushkin A, Lallai V, Fowler JP, Hernandez-Vasquez A, Sherafat Y, and Fowler CD (2020) The Long-Term Effects of Adolescent Nicotine and Cannabinoid Exposure on Nicotine Self-Administration and Relapse-Related Behaviors in a Mouse Model. Joining Forces 2020: Ending the Tobacco Epidemic for All. Palm Desert, CA: Tobacco-Related Disease Research Program Annual Conference. *accepted but conference cancelled due to COVID-19
15. Serrano R, **Dukes AJ**, Pushkin A, Fowler JP, Fowler CD (2019) The Effects of Adolescent Nicotine and Cannabinoid Exposure on Adult Behavior. ABRCMS 2019 Abstract Viewer/Itinerary Planner. Anaheim, CA: Annual Biomedical Research Conference for Minority Students.
16. **Dukes AJ**, Fowler JP, Lallai V, Pushkin AN, and Fowler CD (2019) Differential Nicotine Self-Administration Effects in Adult Mice Following Adolescent Exposure to Nicotine and a Cannabinoid Agonist. 2019 Diversity Poster Session Planner. Chicago, IL: Society for Neuroscience.
17. Hernandez A, **Eugene (Dukes) AJ**, Pushkin A, Fowler JP, Fowler CD (2018) The Effects of Adolescent Exposure to Nicotine and/or a Cannabinoid Agonist on Cognitive and Affective Behaviors in Adult Mice. ABRCMS 2018 Abstract Viewer/Itinerary Planner. Indianapolis, IN: Annual Biomedical Research Conference for Minority Students.
18. **Eugene (Dukes) AJ**, Pushkin AN, Mendoza-Torres A and Fowler CD (2018) Sex-dependent Behavioral Effects of Adolescent Exposure to a Cannabinoid Agonist and Nicotine in Adult Mice. 2018 Abstract Viewer/Itinerary Planner. San Diego, CA: Society for Neuroscience.
19. **Eugene (Dukes) A**, Dixit S, and Harrison F. (2017) Ceftriaxone: Upregulation of GLT-1to Reduce the Effects of Kainic Acid Induced Seizures. 19th Annual Fisk University Research Symposium. Nashville, TN: Fisk University.
20. **Eugene (Dukes) A**, Mallya A, and Deutch A. (2016) Characterization of Glial Proteins during Prefrontal Cortical Development. ABRCMS 2016 Abstract Viewer/Itinerary Planner. Tampa, FL: Annual Biomedical Research Conference for Minority Students.

Research Experience

Summer Program in Neuroscience, Excellence, and Success (SPINES) 2019
Marine Biological Laboratory - Woods Hole, MA
Co-Advisors: Gina Poe, PhD and Carmen Maldonado-Vlaar, PhD

Graduate Student Researcher 2017-2022
Department of Neurobiology & Behavior - University of California, Irvine, Irvine, CA
Principal Investigator: Christie D. Fowler, PhD

Undergraduate Research Assistant 2016-2017
Department of Medicine - Vanderbilt University, Nashville, TN
Principal Investigator: Fiona Harrison, PhD

Course-Embedded Research Assistant 2015-2016
Department of Life and Physical Sciences - Fisk University, Nashville, TN
Principal Investigator: Phyllis Freeman, PhD

Teaching Experience

Research Methods in Psychology Instructor 2021
Department of Psychology – Xavier University of Louisiana

University Studies 83/84 Instructor 2021
Division of Undergraduate Education - University of California, Irvine

Pedagogical Fellow 2021
Division of Teaching Excellence and Innovation - University of California, Irvine

Neurobiology Courses Teaching Assistant 2019-2020
Dept. of Neurobiology and Behavior - University of California, Irvine
N113L: Neurobiology Lab, N121/N233: Neurobio of Addiction and N120A: Human Biology

Anatomy Dissection Lab Instructor 2018-2019
Code Ninjas – Palos Verdes, CA

Science Program Instructor 2018
Boys and Girls Club - Orange Coast and Santa Ana, CA

Laboratory and Teaching Assistant 2015
Fisk University Chemistry and Sociology Departments

Peer Tutor 2014-2017
Fisk University - Academic Excellence and Student Performance (AESP) and Leadership Enrichment and Academic Development (LEAD)

Teaching and Professional Certificates

1. Digital Learning Institute Certificate 2021
2. Certificate in Teaching Excellence 2021
3. Certificate in Course Design 2020
4. Associate at the Center for the Integration of Research, Teaching, and Learning 2020
5. Teaching Assistant Professional Development Program 2018
6. Effective Communication for Scientists Certificate 2018
7. Mentoring Excellence Program Certificate 2018

Guest Lectures

1. **Neurobio N121/N233: Neurobiology of Drug Addiction** Spring 2021
   Nicotine and Cannabinoid Self-Administration
   Course Instructor: Christie Fowler, PhD

2. **BioSci N122: Scientific Argumentation** Winter 2021
   Fallacies of Relevance
   Course Instructor: Audrey Chen Lew, PhD

3. **PhrmSci 42: Life 101** Fall 2020
   Anti-Racism Module: Becoming Anti-Racist
   Course Instructor: Mahtab Jafari, PhD

4. **Neurobio N121/N233: Neurobiology of Drug Addiction** Spring 2020
   Nicotine: Research Articles Discussion
   Course Instructor: Christie Fowler, PhD

5. **BioSci N113L: Neurobiology Lab** Winter 2020
   Neuropharmacology: The Worm Crop-Gizzard
   Course Instructor: Audrey Chen Lew, PhD

Invited Talks

1. Seminar Speaker, "Assessing the Lasting Effects of Adolescent Nicotine and THC Exposure in a Mouse Model", Spring Neuroscience Colloquium Series, University of St. Thomas 2022
2. Speaker, "Using Social Media to Make Connections and Build Community", Diversifying the Community of Neuroscience (CNS), University of Minnesota 2022
3. Keynote Speaker, "Being a Black Woman in Neuroscience and Building #BlackInNeuro", Graduate Women in Science and Engineering Spring Luncheon, Boston University 2022

4. Keynote Speaker, "Being #BlackInNeuro: Experiences as a First-Gen Black Neuroscientist", Neuroscience Undergraduate Research Virtual Symposium (NURVS II), Faculty for Undergraduate Neuroscience 2022
5. Speaker, "Being a Black, First-Gen Neuroscientist", Neuromodulation and Psychiatric Neurosurgery Group "Neuro Week", University of Minnesota 2022
6. Seminar Speaker, "Building the #BlackInNeuro Community as a Graduate Student" University of Louisville School of Medicine 2022
7. Seminar Speaker, "Being #BlackInNeuro: Building a Community" Black Speaker Seminar Series, University of Illinois at Chicago Psychology Dept 2022
8. Seminar Speaker, "The Lasting Impact of Adolescent Nicotine and THC Exposure on Adulthood Drug-Seeking Behaviors" Black Speaker Seminar Series, University of Illinois at Chicago Psychology Department 2022
9. Speaker, "What Does an Addiction Neuroscientist Look Like?" Off the Curriculum London Charity Symposium 2022
10. Seminar Speaker, "Being a Better Mentor and Friend: Suggestions on Supporting Historically Marginalized Students and Colleagues" Diversity and Inclusion Seminar, University of Alabama Birmingham 2022
11. Panelist, "Future Careers and Paths to Becoming a Neuroscientist", Diversity, Equity, and Inclusion Seminar, Wellesley College 2021
12. Speaker, "Leveraging Social Media to Build Your Professional Network and Brand" Pedagogical Fellows Program, University of California, Irvine 2021
13. Keynote Speaker, "Navigating Higher Education as a First-Gen Student", First Generation College Student Celebration, California State University, Long Beach 2021
14. Power Hour Speaker, "Addressing Racial and Gender Disparities in the Cannabinoid Research Community" The GRC Power Hour, Cannabinoid Function in the CNS Gordon Research Conference, Venture, CA 2021
15. Panelist, "What is a Fellowship? – Graduate Panel", Irvine Interdisciplinary Institute in Neuroscience Summer Program, Center for the Neurobiology of Learning and Memory, Irvine, CA 2021
16. Speaker, "Why Black In Neuro?", World Women in Neuroscience Science Education, Virtual Educational Webinar 2021
17. Panelist, "How to Use Twitter to Advance Your Career and Make an Impact" Women In Science, Massachusetts General Hospital and Athinoula A. Martinos Center of Biomedical Imaging, Boston, MA 2021
18. Panelist, "All Hands on Deck: Patching the Leaky Academic Pipeline", Student and Postdoc Special Interest Group for the 2021 Organization for Human Brain Mapping Annual Meeting, Virtual Conference 2021
19. Commencement Speaker, University of California, Irvine School of Biological Sciences, Irvine, CA https://www.youtube.com/watch?v=2j-pMvBGzr8 2021
20. Panelist, "How Communicating Science Through Social Media Builds Community", 2021 Science Communication Week, Yale Biological and Biomedical Sciences Diversity and Inclusion Collective, Yale University 2021
21. Speaker, "Finding Community as the Only One in the Room", Anthropology and Biology Colloquium, Saddleback Community College, Mission Viejo, CA https://www.youtube.com/watch?v=uyucdg8bkAk 2021
22. Speaker, "Being an Addiction Neuroscientist", Brain Awareness Week 2021 2021

Society For Neuroscience Sun City Chapter, El Paso, TX

23. Speaker, "What does an Addiction Neuroscientist look like?", Vaugh International Studies Academy, San Fernando, CA 2021
24. Panelist, "Black In Neuroscience: Being a Better Advocate for Yourself and Others" Neuroscience Institute, Diversity Equity and Inclusion Panel, Georgia State University, Atlanta, GA 2021
25. Speaker, "How to Better Support BIPOC Trainees in STEM and How Students Can Inspire Change", Biomedical Graduate Research Education And Training (BGREAT) Conversations, University of Minnesota, Minneapolis, MN 2021
26. Seminar Speaker, "Becoming an Addiction Neuroscientist as a Low-Income, First-Gen Daughter of Immigrants", Biological Sciences Speaker Seminar Series California State University Long Beach, Long Beach, CA 2021
27. Speaker, "How to be Anti-Racist", DECADE School of Medicine, University of California Irvine, Irvine CA 2021
28. Speaker, "Navigating Safe Spaces & Finding Community", Interdepartmental Neuroscience Program, Irvine, CA 2020
29. Speaker, "Life as a Neuroscientist", Avalon Carver Community Center Youth Summer Program, Los Angeles, CA 2020
30. Panelist, "Applying to Graduate School During a Pandemic", Trailblazer in STEM, University of California, Irvine Graduate Division and Office of Access & Inclusion, Irvine, CA 2020
31. Speaker, "Becoming Anti-Racist: Being a Better Advisor, Lab Mate and Friend to Black Colleagues", University of California, Irvine 2020
32. Speaker, "Vaping Nicotine and Cannabis: An Adolescent Epidemic with Long-Lasting Effects", Brews and Brains, Irvine, CA 2019
33. Speaker, "Nicotine, THC, and Me: The Long-Term Effects of Adolescent Nicotine and Cannabinoid Use", Cool Science Café hosted by the MARC U*STAR program, Fisk University, Nashville, TN 2019
34. Speaker, UCI Brilliant Future Campaign – Fellowship Recipient Perspective, Graduate Division, University of California, Irvine 2019
35. Panelist, "From an HBCU to a UC PhD student", UC-HBCU UCOP Program, University of California, Irvine 2019
36. Panelist, Graduate Student Experience, California State University Long Beach BUILD program, Graduate Division, University of California, Irvine 2019
37. Panelist, NSF-GRFP Student Experience, Graduate Division, University of California Irvine Irvine, CA 2019
38. Speaker, "#GradLife: My Perspectives as a Black Female Neuroscientist" Departments of Biology and Biochemistry, Fisk University, Nashville, TN 2019
39. Panelist, "Why Graduate School?", Diverse Educational Community And Doctoral Experience, University of California, Irvine 2018
40. Panelist, NSF-GRFP Student Experience, School of Biological Sciences, University of California, Irvine Irvine, CA 2018
41. Panelist, NSF-GRFP Student Experience, Graduate Division, University of California Irvine Irvine, CA 2018
42. Speaker, "Getting a College Degree without Student Loans" Centennial High School, Compton Unified School District, Los Angeles, CA 2017

Honor and Professional Society Memberships

Faculty for Undergraduate Neuroscience	2021
Black In Neuro	2020
Society for Research on Nicotine and Tobacco	2018
Society for Neuroscience	2018
National Center for Faculty Development & Diversity	2018
Phi Beta Kappa Academic Honor Society	2017
Beta Kappa Chi Scientific Honor Society	2017
Mortar Board National College Senior Honor Society	2015
Alpha Mu Gamma Foreign Language Honor Society	2014

Leadership Activities

President – Black in Neuro Executive Committee	2020 - Present
DECADE PLUS Mentor – UCI Graduate Division	2020
DECADE Student Rep. – Department of Neurobiology & Behavior	2019-2021
CNLM Ambassador – K-12 Committee	2019-2021
Competitive Edge Peer Mentor – Graduate Division	2019
CNLM Ambassador – Professional Development Committee	2018-2019
Graduate Student Representative - INP Executive Committee	2018-2020
Center for Neurobiology Learning and Memory (CNLM) Ambassador	2018-2021

Professional Service

Selection Committee - Irvine Summer Internship in Neuroscience Program	2022
Selection Committee – UCI Pedagogical Fellows Program	2021
Black Neuro Intersections Day Organizer – Black In Neuro Week 2021	2021
Vanguard STEM's Guerilla Mentoring Session at the STEMNoire conference	2021
Graduate Student Representative - UCI Biological Sciences Climate Council	2021
Session Chair - International Behavioral Neuroscience Society Annual Meeting	2021
Selection Committee - CNLM Summer Internship in Neuroscience Program	2021
Session Chair - Society for Research on Nicotine and Tobacco Annual Meeting	2021
Pedagogical Liaison – Department of Neurobiology and Behavior	2020
UCI School of Biological Sciences Inclusive Excellence Task Force	2020
Lead Organizer – Inaugural Black in Neuro Week	2020
Session Chair - Society for Research on Nicotine and Tobacco Annual Meeting	2020
NSF GRFP writing consultant - School of Biological Sciences	2019
Organizing Committee - Irvine Center for Addiction Neuroscience Symposium	2019
Graduate Experience Panel Facilitator - Interdepartmental Neuroscience Program	2019
Oral Presentation Skills Assistant – Competitive Edge Summer Research Program	2019
Poster Presentation Assistant – UCI Summer Undergraduate Research Program	2019
Writing Mentor – UC-HBCU Chemistry at the Space-Time Limit Summer Program	2019
Recruitment Weekend Coordinator – Interdepartmental Neuroscience Program	2019

Graduate Student Recruiter – Annual Biomed Research Conf for Minority Students	2018
Graduate Student Recruiter - Society for Neuroscience	2018
NSF GRFP writing consultant - School of Biological Sciences	2018
Graduate Panel Facilitator - Interdepartmental Neuroscience Program	2018
Session Co-chair – Irvine Center for Addiction Neuroscience Symposium	2018

Media

1. Invited LIVE radio guest *"I'm Only One Person"* interviewed by Claudia Shambaugh. March 1, 2022. KUCI Ask A Leader Segment. https://askaleader.com/?p=2537
2. Interviewed via Instagram Live, *Black In Neuro and Diversity in Science* by Tariro Hlahla and Lilo Noort. February 18, 2022. Carleton STAR and the CHAIM Centre. https://www.instagram.com/tv/CaI2Eoqg2Vj/?utm_medium=copy_link
3. Invited podcast guest speaker *"Legacy"* interviewed by Asma Bashir. February 5, 2022. Her Royal Science Podcast. https://www.herroyalscience.com/podcast/episode/9050032b/29-legacy
4. Invited podcast guest speaker, *"Rejection just means Redirection"* interviewed by JP Flores. January 28, 2022. From Where Does It STEM? Podcast. https://open.spotify.com/episode/08BuMlS2iGBizOvUbt8Cv8
5. Interviewed during *The Welcome Table with Sydney and Tatum.* May 3, 2021. UCI's Black Thriving Initiative under the Office of Inclusive Excellence. https://www.youtube.com/watch?v=dRc0SoOWc3Y
6. Interviewed for *UC Irvine Student's Black In Neuro Efforts Garner Worldwide Participation* by Ruksana Hussain. February 26, 2021. Irvine Weekly. https://irvineweekly.com/uc-irvine-students-black-in-neuro-efforts-garner-worldwide-participation/
7. Interviewed for *#BlackInNeuro: How a Hashtag Forged Community for Black Scientists* by Amanda D'Ambrosio. February 25, 2021. MedPage Today. https://www.medpagetoday.com/special-reports/exclusives/91355
8. Interviewed during *Stat+ Conversations: A conversation on race, science, and advocacy with Black In Neuro founder Angeline Dukes* by Nicholas St. Fleur. February 16, 2021. Stat News. https://jwp.io/s/EKaic21M
9. Interviewed for *The Sky's the Limit for Black In Neuro* by Diana Kenney. February 5, 2021. The Well: MBL News from the Source. https://social.mbl.edu/the-skys-the-limit-for-black-in-neuro
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Conferences and Symposiums

60th Annual Meeting of the American College of Neuropsychopharmacology	2022
Cannabinoid Function in the CNS Gordon Research Conference and Seminar	2021
International Behavioral Neuroscience Society 30th Annual Meeting	2021
Virtual Center for Neurobiology Learning & Memory Spring Conference	2021
Virtual Conference: Society for Research on Nicotine and Tobacco Annual Meeting	2021
Society for Neuroscience Global Connectome	2021
59th Annual Meeting of the American College of Neuropsychopharmacology	2020
First Annual Black In Neuro mini-conference	2020
10th Annual Society for the Advancement of Biology Education Research Meeting	2020
Society for Research on Nicotine and Tobacco 26th Annual Meeting	2020
The 49th Annual Society for Neuroscience Conference	2019
Irvine Center for Addiction Neuroscience Research Symposium	2019
Summer Program in Neuroscience, Excellence, and Success Annual Symposium	2019
Center for Neurobiology Learning & Memory Annual Meeting	2019
2019 North American Cannabis Summit	2019
Annual Biomedical Research Conference for Minority Students	2018
The 48th Annual Society for Neuroscience Conference	2018
Irvine Center for Addiction Neuroscience Research Symposium	2018
UC Irvine International Conference on Learning and Memory	2018
19th Annual Fisk University Research Symposium	2017
14th Annual Tennessee LSAMP Undergraduate Research Conference	2017
Annual Biomedical Research Conference for Minority Students	2016
Vanderbilt Summer Science Academy Research Symposium	2016
Fisk University Research Symposium	2016

Outreach

STEM Career Series – South High Community School	2021
Ask Me Anything Panel – Westminster High School	2021
Women's History Month Community Program - Watts Healthcare	2021
Annual Saturday Science Fun Day Instructor - Friendship Baptist Church	2020
Youth Village Science Booth Coordinator – Orange County Black History Fair	2020
Girls' STEM Night - MacArthur Fundamental Intermediate School	2019
Annual Saturday Science Fun Day Instructor - Friendship Baptist Church	2019
Addiction Neuroscience Lesson Facilitator – Alliance Luskin Academy	2019
Girls in STEAM Conference - University of California, Irvine CaSTL Center	2019
Nicotine Addiction Session Presenter – REMIND UCI	2019
Tobacco Use Workshop presenter – MacArthur Fundamental Intermediate School	2019
Free Public Neuroscience Workshop – Newport Beach Public Library	2019
Backyard Brains Presenter – Culverdale Elementary	2019
College Booth Volunteer – Orange County Black History Fair	2019
Galentine's Day STEM Volunteer – MacArthur Fundamental Intermediate School	2019
Irvine Unified School District Science Fair Judge	2019
Girls' STEM Night - MacArthur Fundamental Intermediate School	2018
Annual Saturday Science Fun Day Instructor - Friendship Baptist Church	2018
Annual Ask-a-Scientist Night - Irvine Unified School District	2018
Science Booth Volunteer - McGaugh-Gerard Inaugural Lecture	2018
San Diego Festival of Science and Engineering Volunteer	2018
Youth Science Booth Volunteer – Orange County Black History Fair	2018
Annual Saturday Science Fun Day Instructor - Friendship Baptist Church	2017

ABSTRACT OF THE DISSERTATION

Drug Addiction - the Research & the Researchers:
The Lasting Impact of Adolescent Nicotine and Cannabinoid Exposure
and
The Importance of Effectively Supporting Historically Marginalized Scientists
by
Angeline Joan Dukes
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University of California, Irvine, 2022
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In the past decade overall, there have been increases in adolescent nicotine and cannabinoid use. Yet the long-term implications of this drug exposure, in particular the co-exposure of both of these drugs, on cognition, reward-related behaviors, later drug intake, and relapse-related behaviors is largely understudied. This dissertation explores the novel studies conducted to assess the long-term implications of adolescent nicotine and cannabinoid exposure. Using various behavioral paradigms and intravenous nicotine self-administration in a mouse model, we have shown that adolescent exposure to a cannabinoid or co-exposure to both nicotine and a cannabinoid alters anxiety-related behaviors, cognitive flexibility, natural reward consumption, and nicotine intake in a sex-dependent manner (Pushkin et al. 2019 and Dukes et al. 2020). Moreover, we have shown that adolescent drug exposure can alter the responsivity to cue-induced drug seeking later in life (Dukes et al. 2022 *in prep*). The final chapter of this dissertation branches off into a more global perspective focusing on the importance of proper mentorship and support for people from historically marginalized backgrounds in the field of neuroscience (Dukes 2020, Singleton et al. 2020, and Singleton et al. 2021).

INTRODUCTION

Substance use disorder is typically characterized by excessive and compulsive drug seeking behaviors, in which the individual continues to use the drug despite harmful consequences. Worldwide, nicotine is the leading cause of preventable death and cannabis is the most abused illicit substance [1, 2]. Following initial experimentation, continued use may lead to future patterns of abuse. Thus, tobacco use disorder and cannabis use disorder represent the consequences of dependence on either nicotine or an exogenous cannabinoid, respectively.

Tobacco and Nicotine

Over 1.3 billion people around the world report using tobacco products, which includes cigarettes, cigars, e-cigarettes, and smokeless tobacco [2]. Nicotine is the main psychoactive component derived from the leaves of the tobacco plant, *Nicotiana tabacum*. Nicotine use in humans can lead to multiple positive effects [3], including mild euphoria, decreased appetite, reduced stress/anxiety, and improvements in memory and concentration. In addition to nicotine, tobacco smoke contains many other toxic chemicals, such as ammonia, arsenic, formaldehyde, acetaldehyde, and tar [4]. Thus, it is perhaps not surprising that tobacco smoking has been causally linked to multiple types of cancer, stroke, coronary heart disease, lung disease, chronic obstructive pulmonary disease and periodontitis [5]. In the US alone, the combined direct cost of healthcare to treat smoking-related disease and the human capital losses amass to more than $300 billion each year [6].

In the past decade, there has been the emergence of a potentially safer alternative to tobacco cigarettes, electronic nicotine delivery systems (ENDS). ENDS heat liquid to produce an aerosol that users inhale, and like tobacco smoke, allows for nicotine absorption through the lungs. In recent years overall, tobacco cigarette use has been declining, but the use of ENDS conversely increased [7]. In 2011, it was estimated that there were seven million ENDS users worldwide, but by 2018, the number dramatically increased to 41 million. ENDS have been beneficial as a therapeutic approach to assist individuals in reducing the number of tobacco cigarettes smoked. For instance, in one study, over 80% of former tobacco smokers reported that ENDS helped them quit smoking tobacco cigarettes [8]. However, ENDS use by individuals who have never smoked tobacco cigarettes remains a major concern, due to the increased potential of developing nicotine dependence and later tobacco cigarette use [9]. Indeed, of the estimated 5.6 million US adults who currently used ENDS, 1.3 million of them were never smokers [8]. Moreover, of those that were dual users of both tobacco cigarettes and e-cigarettes, 70% of them reported trying to use ENDS to quit smoking, which then led to dual use [8]. Although ENDS are considered to be safer than tobacco cigarettes, ENDS can omit harmful constituents, including carcinogens, nickel, and lead [10]. Further, to appeal to youth, additives in the ENDS solutions may flavor the vapor as candy or fruit, but these flavoring chemicals may lead to adverse health consequences when inhaled.

The vast majority of adult smokers began smoking during adolescence [10]. Each day, approximately 2,000 youth smoke their first cigarette and over 300 become daily smokers [11]. Previous studies have shown that the younger people are when they begin using tobacco products, the more likely they are to develop nicotine dependence and other substance use disorders [12]. It has also been shown that adolescents can become nicotine

dependent very quickly, even after occasional intermittent use [13]. Furthermore, a meta-analysis of longitudinal studies confirms that ENDS use is associated with an increased likelihood of future cigarette smoking [14]. Although the percentage of teen ENDS use had been consistently increasing over the years, the percentage of high school seniors vaping nicotine actually decreased from 34.5% in 2020 to 26.6% in 2021 during the COVID-19 pandemic in the US [15]. This decrease in adolescent drug use was surprisingly consistent across many substances, including alcohol and opioids. This phenomenon could possibly be attributed to limited access to drugs during government 'stay at home' orders, reduced in-person peer pressure, increased messaging of the harmful effects of drug products targeted at youth, and/or potential survey response biases as adolescents are likely responding in the presence of a parent or guardian at home.

Cannabis, THC, and Spice

According to the World Health Organization, cannabis, also known as weed or marijuana, is the most abused illicit drug [1]. As of 2021, only eight countries have at least partially legalized cannabis for recreational use, but approximately 200 million people report using cannabis worldwide [1]. Cannabis is derived from the *Cannabis* plant which contain over 100 compounds called phytocannabinoids, with Δ^9-tetrahydrocannabinol (THC) and cannabidiol being the most well-characterized. Cannabis use may induce sensations of euphoria, altered sense of time, distorted sensory/body perception, and mood changes [16]. THC is the main psychoactive component in cannabis and can result in feeling 'high', along with increased anxiety and paranoia, altered perception, impaired working memory, slower movements, and cognitive deficits, depending on the dose [16, 17]. Among

youth, smoking cannabis appears to be becoming somewhat less prevalent, but this downward trend has been paralleled by a general upward trend in consumption of edibles and use of THC vapes [18]. Of concern, high frequency adolescent cannabis use has been linked to deficits in attention, learning, and memory [19], as well as mental health issues including increased depression, anxiety, suicidal ideation and schizophrenia [20, 21]. Similar to nicotine, however, in correlation to the COVID-19 pandemic, there was a decrease in the percentage of adolescents who report using cannabis in 2021 [15]. Interestingly, there is evidence that low doses of THC in older mice may help restore cognitive function [22]. Similarly, in older humans (age 65 and above), short-term low-dose cannabis consumption does not seem to have adverse effects on cognition and can aid in pain management [23]. THC can be also used to help counteract weight loss in HIV/AIDS patients and to alleviate nausea for patients undergoing chemotherapy [24].

Synthetic cannabinoids are those created in a laboratory and fall under the drug classification 'New Psychoactive Drugs'. Examples include WIN 55,212-2 (WIN) and CP-55,940 – which are compounds that can be used in the street drug termed 'spice'. Some forms of spice are created by spraying synthetic cannabinoids onto shredded plant material, with users smoking the resulting combination like a joint. However, it has more intense psychoactive and physiological effects than THC [25]. Synthetic cannabinoids were initially created for pharmaceutical research and were not intended for human consumption. However, because synthetic cannabinoids have similar physiological effects as THC and were not federally illegal, they have been sold in US and European street drug markets since the early 2000s. Further, these synthetic cannabinoids are not typically tested for during routine drug screens and often remain undetected when law enforcement or hospitalization is

involved. Synthetic cannabinoids induce many more intense effects than merely altering one's mood and perceptions, such as tachycardia, psychosis, hallucinations, respiratory distress, and in some cases, death [26, 27]. In 2012, 26 different formulations of spice were banned with the Synthetic Drug Abuse Prevention Act in the US, classifying them as Schedule I drugs. Nevertheless, as different versions of these synthetic compounds become illegal, new modified versions created by underground chemists consistently reappear in the market. They have not yet been classified as illegal, but still can be quite harmful.

Drug co-use is also of concern, given the potential synergistic effects on the user. For instance, a study in youth aged 12-17 found that those who used cannabis were more likely to use nicotine products at the same time, or initiate nicotine consumption within one year [28]. Daily cannabis use has also been associated with co-use of opiates, cocaine, and/or inhalants [29], and approximately half of young cannabis users report simultaneously consuming alcohol and cannabis [30]. Moreover, young adults co-using tobacco and cannabis were more likely to use nicotine ENDS, cocaine, and greater amounts of cannabis than those that just consumed cannabis alone [31]. In older adults, using cannabis is associated with an increased likelihood of being diagnosed with a substance use disorder for either nicotine, alcohol, or cannabis [23]. Thus, the drug co-use condition represents a significant health concern among various age groups.

Brain Mechanisms Underlying Drug Use

When inhaled, nicotine readily enters the bloodstream through the alveoli in the lungs and becomes absorbed into the brain within seconds. Nicotine selectively binds to nicotinic acetylcholine receptors (nAChRs), which are pentameric ligand-gated ion channels

located on either the presynaptic or postsynaptic membrane [3]. The nAChR subtype may be heteromeric or homomeric, containing a combination of α and β subunits or containing all the same subunit, respectively. The α subunits present in nine different types, α2 - α10; whereas the β subunits present in three different types, β2 - β4. Various combinations of these subunits result in diverse effects on the pharmacokinetics of the receptor with ligand binding. The α7, α4 and β2 subunits are the most prevalent in the central nervous system. The homomeric α7 nAChR has a relatively lower affinity for nicotine and is important for modulating inflammation [32]. The α4 and β2 subunits combine to form a functional heteromeric receptor with a high affinity for nicotine; the α4β2 nAChR is involved in mediating nicotine's reinforcing and rewarding properties through receptor localization in the mesolimbic circuit [33]. Further, receptors containing the α5, α3 and β4 subunits have been shown to modulate aversive signaling that limits nicotine intake and aspects of the withdrawal syndrome via receptor localization in the habenulo-interpeduncular circuit [3].

Like nicotine, when cannabis smoke is inhaled, active phytocannabinoids pass from the lungs into the bloodstream and are carried throughout the brain and body. THC acts on the cannabinoid 1 receptor (CB1R) and cannabinoid 2 receptor (CB2R) [34]. In the brain, CB1Rs are mainly localized in neurons and astrocytes, whereas CB2Rs are primarily found on immune cells. In humans and rodents, CB1Rs are highly expressed on neurons in the neocortex, hippocampus, amygdala, cerebellum, and basal ganglia [34, 35]. Since endogenous cannabinoids engage in retrograde signaling from the cell body to the presynaptic axon terminal, activation of CB1Rs results in inhibition of presynaptic neurotransmitter release [34]. CB1Rs are also located on postsynaptic membranes and astrocytes [34]. Of note, THC is a partial agonist of the CB1Rs and CB2Rs, whereas synthetic

cannabinoids are typically full agonists. Cannabinoids can also act on other receptors, including GPR55 and TRPV1, although the functional role of these receptors is lesser known.

When individuals co-use nicotine and cannabinoids, one would expect activity at both the nAChRs and CB1Rs. Both of these receptor types exhibit overlapping expression patterns in drug addiction-associated brain regions, such as the prefrontal cortex, nucleus accumbens (NAc), ventral tegmental area (VTA), and amygdala [36, 37]. In particular, the mesolimbic dopamine pathway, a circuit from the VTA to the NAc, controls reward processing and the reinforcement of natural rewards and most drugs of abuse. Mechanistically, as nicotine and cannabinoids bind to nAChRs and CB1Rs in the VTA, they increase the firing rate of dopamine neurons and trigger the release of dopamine to the NAc which subsequently reinforces the drug-taking behavior. Nicotine acts on the $\alpha 4\beta 2$-containing nAChRs to mediate dopamine signaling via their locations on both dopaminergic and GABAergic neurons in the VTA and neuron terminals in the NAc [38]. Due to a lack of CB1R expression on VTA dopamine neurons, cannabinoids likely act on this circuit indirectly [39]. Through retrograde mechanisms, cannabinoid binding to presynaptic CB1Rs expressed on GABAergic presynaptic terminals decrease GABAergic inhibition, thereby increasing dopamine release in the NAc [39, 40]. In addition, it is important to acknowledge the involvement of other neurotransmitters, such acetylcholine, glutamate, and serotonin in consideration of the intricate complexity of the projections among brain regions. Thus, nicotinic and cannabinoid signaling within reward-related brain regions may lead to interactions among signaling mechanisms that modulate various aspects of drug-taking behaviors.

Impact of Cannabinoids on Cognition, Behavior and Nicotine Use

Approximately 20-30% of people who experiment with cigarettes will meet criteria for nicotine use disorder during their lifetime [41]. Around 30% of cannabis users are anticipated to develop some degree of cannabis use disorder [42]. Individuals who begin using cannabis prior to 18 years of age have a four to seven times increased likelihood of developing the use disorder [43]. Moreover, as noted above, the co-use of both substances is quite frequent. Around 60% of cigarette smokers reported ever using cannabis, and 90% of cannabis users reported ever smoking cigarettes in their lifetime [44]. The rates of daily cannabis and nicotine co-use have doubled from 2002 to 2014 in the US [45]. While individual drug use has its own potential to develop into a substance use disorder, co-users of both substances are at an increased risk of developing both nicotine dependence [44] and cannabis dependence [46]. This increased risk is of further concern because adults with co-occurring cannabis use disorder and nicotine dependence are more likely to have bipolar, anxiety, and personality disorders than those with only nicotine dependence [47].

Among youth and young adults, the concern of these co-occurring substance use disorders is also prevalent. Teens who vape nicotine or smoke hookah are four times more likely to start smoking cannabis within two years [48], and current adolescent tobacco smokers who frequently use cannabis are more likely to report nicotine dependence [49]. Likewise, adolescents who use cannabis are more likely to become daily cigarette smokers and develop nicotine dependence [50, 51]. Additionally, a longitudinal study looking at the trajectories of nicotine and cannabis vaping from adolescence into early adulthood revealed that those who frequently vape were also very likely to be users of both substances [52]. Adolescent and young adult cannabis and tobacco cigarette co-users exhibit increased

cannabis use disorder symptoms including continued use despite negative consequences, developing a tolerance, and inability to reduce cannabis use, in comparison to those who only use cannabis [53]. Moreover, young adults who co-use cannabis and nicotine together report consuming more cannabis and nicotine in the past year than those who only use one of the drugs [54]. In sum, across age ranges, nicotine and cannabis co-use conditions increase the risk of individual drug use and the development of substance use disorders.

Furthermore, sex differences have emerged with the prevalence and patterns of substance use disorders in the population. Adult men are more likely to initiate drug use, but adult women develop substance use disorders more rapidly [55]. Interestingly, during adolescence, drug use is initiated at similar rates between sexes, but boys appear to escalate their drug use faster than girls [56]. According to a recent report, men are more likely to smoke cigarettes, vape nicotine, use smokeless tobacco, and be daily cannabis users [57]. Women with a history of cannabis use are four times more likely to become regular cigarette smokers and almost three times as likely to develop nicotine dependence [58]. However, women report experiencing more intense nicotine withdrawal symptoms [59].While the above findings suggest an important role of age or sex in nicotine and cannabis co-use, many individual, societal, and familial factors also influence drug taking behaviors in humans, and as such, animal models are important to delineate the precise effects of such factors on drug-taking behaviors.

Findings from rodent models have established that cannabinoids and nicotine exert unique effects on drug-related behaviors based on the duration and timing of exposure, in addition to the animal's sex. Interestingly, adolescent male and female rats will self-administer greater amounts of nicotine than adults [60, 61], and nicotine exposure during

adolescence can lead to increased self-administration of other drugs of abuse, including alcohol, methamphetamine, and cocaine [62]. Drug exposure during adolescence can also induce long-lasting effects on the animal into adulthood. Adolescent male rats exposed to THC were found to self-administer higher levels of the synthetic cannabinoid WIN or heroin in adulthood [63, 64]. Furthermore, female rats that were permitted to self-administer nicotine beginning in later adolescence exhibited higher levels of nicotine intake compared to those that initiated self-administration in adulthood [60]. Thus, the stage of development when nicotine and cannabinoid exposure occur as well as the duration of the exposure are important factors that impact later drug-taking.

Cannabinoid and nicotine co-exposure in adulthood also appear to alter later drug-related behaviors. Of further interest, while WIN exposure decreased nicotine self-administration in adult male rats at a moderate nicotine dose, this effect was reversed when the level of effort required to obtain drug infusions was increased under a progressive ratio schedule of reinforcement [65]. Similarly, under operant conditions requiring high levels of behavioral effort, a brief history of THC administration in adulthood increased subsequent nicotine self-administration in male rats [66]. Thus, in high effort situations, cannabinoid exposure can drive an increase in effort to obtain nicotine. Finally, cannabinoid signaling may also be involved in cue-associated nicotine seeking. Male rats administered WIN prior to a cue-induced reinstatement session exhibited increased nicotine-seeking behavior [65]. This suggests that acute cannabinoid receptor activation heightens the responsivity to cues in triggering reward-seeking behaviors. Taken together, these studies highlight the importance of prior drug history at varying developmental stages and level of effort required on the effectiveness of cannabinoids in modulating nicotine reinforcement.

Nicotine and/or cannabinoid use may also alter cognitive and emotion-associated behaviors, which are often correlated with substance use disorders. Acute cannabinoid or nicotine exposure has been shown to induce either anxiolytic or anxiogenic effects dependent on dose, age, or sex [37, 67, 68]. For example, nicotine decreased anxiety-associated behaviors in adolescent male rats, but paradoxically increased anxiety-associated behaviors in females [69]. Further, male and female adolescent rats exposed to cannabinoids exhibited a decrease in short-term and spatial working memory but an increase in depressive-like behaviors [37]. In a study assessing chronic co-exposure of nicotine and the synthetic cannabinoid CP 55,940, both male and female adolescent rats developed increased anxiety-like behavior that was further reflected physiologically by elevated corticosterone, a stress-associated hormone [70]. In contrast, in adult mice, chronic co-exposure to both nicotine and THC decreased anxiety-like behaviors [71]. Similarly, nicotine treatment can reduce some of the anxiogenic effects of acute THC exposure, and THC treatment can attenuate the anxiogenic effects of acute nicotine exposure [68, 72].

Finally, nicotine and/or cannabinoids can induce a significant developmental impact on cognitive outcomes when consumed during pregnancy. Chronic *in utero* exposure to nicotine, THC, or co-exposure to both drugs has been associated with long-term effects into adolescence [73]. Specifically, adolescent male and female rats exposed prenatally to THC exhibited deficits in short-term memory [73]. Interestingly, the adolescent male rats with a prenatal history of nicotine and THC co-exposure exhibited similar deficits in short-term memory, as well as a deficit in pre-pulse inhibition [73], a behavioral outcome associated with schizophrenia symptomology. It is worthwhile to note that the nicotine and THC prenatal co-exposure condition only induced memory-related effects in the males but not

females [73], suggesting that nicotine may have buffered the effects of THC on the developing female brain. Together, these findings indicate that nicotine and cannabinoids induce complex interactions on the brain across various stages of development.

Nicotine and Cannabis Cessation

Less than 10% of those who want to quit smoking cigarettes are successful in the long-term [75]. Most people attempt to quit 'cold-turkey', without the help of any nicotine replacement therapies (NRT), other pharmacotherapies, or behavioral support programs. Unfortunately, this cold-turkey approach induces significant nicotine withdrawal symptoms, such as cravings, irritability, difficulty concentrating, headaches, and insomnia, which can promote relapse as the user attempts to alleviate symptoms with drug re-exposure. By using NRTs, such as nicotine patches, lozenges, or gum, the success of quitting increases to 50-60% at the six-month time point [76]. This is likely due to smokers being able to obtain nicotine from a source other than cigarettes, thereby reducing withdrawal symptoms and easing the transition to abstinence. ENDS were also developed as a type of NRT for adult smokers. It was proposed that this method of administration may be more successful given that the same physical and sensory cues are present as with smoking cigarettes, such as raising the hand to the mouth and inhaling/exhaling smoke. In 2014, 4% of adults in the US reported using ENDS for cigarette cessation, but by 2018, the percentage decreased to 3.2% [7]. Furthermore, about half of adults who vape nicotine also smoke tobacco cigarettes, a behavior known as 'dual use' [77]. Surprisingly, a recent study found that people who quit smoking for more than a year have an increased risk of relapse if they vape nicotine during that time [78]. Additionally, there is increasing evidence of cannabis use in vaping devices

among teens and adults. People who use THC vapes report a high incidence of tobacco product use as well [79]. As such, the absolute effectiveness of ENDS for tobacco cessation remains to be determined. Regardless of the smoking cessation tools implemented, high rates of nicotine relapse remain prevalent.

Modulation of the cannabinoid receptor has also been employed as a novel approach for smoking cessation. In rat models, CB1R antagonists were shown to decrease nicotine self-administration and reduce nicotine-induced dopamine release in the NAc [80], which then led to the progression along the drug development pipeline. Two different CB1R antagonists, rimonabant and taranabant, underwent clinical trials for smoking cessation and were found to be marginally effective [81, 82]. However, both drugs have now been withdrawn from the market due to adverse psychological side effects in humans, including increased anxiety and depression. Cannabidiol, a CB1R and GPR55 antagonist and CB2R reverse agonist, has also been assessed as a modulator for nicotine withdrawal symptoms in a pre-clinical study. Co-exposure to cannabidiol during chronic nicotine exposure reduced the somatic signs of nicotine withdrawal, including paw tremors, head shakes, jumps, and abdominal contractions, in rats [83], suggesting that cannabidiol may be a potential therapeutic in future clinical studies.

Individuals with cannabis use disorder exhibit similar withdrawal symptoms as nicotine, including increased irritability, aggression and depression, sleep difficulty, and physical symptoms (e.g., tremors, chills, and/or headaches) [84]. Indeed, daily cannabis users who attempted to quit report similar withdrawal symptom severity as daily cigarette smokers attempting to quit [85]. A preliminary study has shown that synthetic cannabinoids, such as nabilone, may be used to attenuate these withdrawal symptoms, which was

demonstrated in a small sample of cannabis users in a clinical setting [86]. Targeting nAChRs may also be effective as a treatment for cannabis use disorder. A pre-clinical study in rats showed that blocking α7 nAChRs with a selective antagonist, methyllacotinine, reduced self-administration of the synthetic cannabinoid WIN and prevented THC from increasing dopamine in the NAc shell [87]. This is quite promising because this putative therapeutic did not result in any depressant or toxic effects [87]. More recently, nicotine patches have been examined for alleviation of cannabis withdrawal symptoms. A low-dose nicotine patch was shown to reduce negative affective withdrawal symptoms in subjects that were not heavy tobacco users, but a side effect of nausea was also observed [84]. Importantly, in consideration of the co-use condition, adult tobacco smokers who also smoke cannabis are twice as likely as non-cannabis users to continue smoking tobacco even years later [88]. This could be due to the cannabinoids enhancing the effects of nicotine-associated cues in reinstating the drug-seeking behavior after a quit attempt [65]. However, one study found that people attempting to quit or reduce cannabis intake also report using less tobacco on abstinent days [89]. Thus, research on effective cessation methods for co-users is heavily understudied and needs to be conducted to aid in the smoking cessation of people suffering from co-occurring cannabis use disorder and nicotine use disorder.

Incubation of Drug Craving

People battling with nicotine, cannabis, or co-occurring substance use disorders may try to quit taking the drugs, but the risk of relapse is quite high as most people begin smoking again within the first week [90]. Relapse can occur due to trying to alleviate the negative withdrawal symptoms or it can be triggered by things like stress, acute exposure to the drug,

or certain cues that were previously associated with drug-taking [91, 92]. Cues can include the physical environment, people with whom the drug taking typically occurs, as well as any associated auditory, visual, olfactory, or tactile signals [93]. In animal models, this phenomenon is known as incubation of drug craving in which cue-induced drug-seeking behavior increases over time during abstinence after drug self-administration [94]. In other words, a rodent is allowed to intravenously self-administer a drug of abuse for a while, such as cocaine or nicotine, then the drug is taken away. During this abstinence period, when the rodent is put back in the same environment with the same sensory cues as when receiving the drug, they will actively seek it more. This active drug seeking is usually measured in lever pressing or nosepokes (i.e. the action that the animal previously had to perform to obtain the drug). The cue-induced portion of this paradigm is highly pertinent as it is the visual, auditory, and olfactory cues that trigger this drug-seeking behavior. This phenomenon was first seen in humans experiencing progressively higher rates of cue-induced cocaine craving and cigarette craving during the first few weeks of abstinence [95, 96]. The incubation of drug craving effect has now been replicated in rodent models using a wide variety of drugs of abuse, including heroin, alcohol, and nicotine [94, 97]. Understanding how prior drug history might impact this cue-induced drug-seeking behavior can help create more effective relapse interventions for those in the early stages of abstinence.

Diversity, Equity, and Inclusion

Beyond the research itself, it is important to have insight into the scientists conducting the research, the populations being studied, the dissemination of this new scientific knowledge, as well as the people being impacted by the findings. The final chapter

of this dissertation explores a broader perspective of these crucial issues and the necessity of more support for people from historically marginalized backgrounds in the field of neuroscience at every level. Publications within this chapter include discussions on Black, Indigenous, and Hispanic early career scientists being cited less often, receiving less grants, having fewer authorships, and receiving lower salaries while still doing the majority of diversity, equity, and inclusion work to make academia more accessible for subsequent generations [98, 99]. This section also discusses the high attrition of trainees from disadvantaged backgrounds as well as the need for stronger community, professional resources, and culturally competent mentors to advocate on their behalf [100-102]. Finally, it delves into the necessity of representation and accountability in the scientific community as well as the systemic issues that inhibit this progress [103].

Valuing diversity, equity, and inclusion principles, not only makes academia more welcoming while bringing in a wealth of knowledge and unique perspectives, but it also strengthens the research being done. In fact, studies have shown how productivity, innovation and the success of research is enhanced when these ideals are embraced [104]. Furthermore, it allows those researchers to then disseminate information effectively about critical research progresses to their own communities while conducting meaningful outreach and mentoring the next generation of researchers.

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Chapter 1: Cannabinoid and nicotine exposure during adolescence induces sex-specific effects on anxiety- and reward-related behaviors during adulthood

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ABSTRACT

Nicotine and cannabis use during adolescence has the potential to induce long lasting changes on affective and cognitive function. Here, we examined whether adolescent exposure to nicotine, the cannabinoid agonist WIN55-212,2 (WIN), or co-exposure to both would alter operant learning, locomotion, and anxiety- and reward-related behaviors in male and female mice during adulthood. Males exposed to a moderate dose of WIN (2 mg/kg) or co-exposed to nicotine and the moderate dose of WIN exhibited decreased anxiety-associated behaviors and increased cognitive flexibility, but did not differ in operant learning or generalized locomotion. In contrast, differences were not found among the females in these measures at the moderate WIN dose or in both sexes with exposure to a low WIN dose (0.2 mg/kg). Furthermore, a sex-dependent dissociative effect was found in natural reward consumption. Males exposed to the moderate dose of WIN or co-exposed to nicotine and the moderate dose of WIN demonstrated increased sucrose consumption. In contrast, females exposed to the moderate dose of WIN exhibited a decrease in sucrose consumption, which was ameliorated with co-administration of nicotine. Together, these novel findings demonstrate that adolescent exposure to cannabinoids in the presence or absence of nicotine results in altered affective and reward-related behaviors during adulthood.

INTRODUCTION

Tobacco smoking results in millions of preventable deaths each year worldwide. Nicotine, the main psychoactive component in tobacco, is considered to be responsible for the development and maintenance of dependence in humans. Nicotine's effects on adolescent development have become of increasing concern given the emergence of e-cigarettes, which deliver vaporized nicotine [1]. According to a nationwide CDC survey, ~30–45% of high school students self-reported prior use of cigarettes, vaporized nicotine products, and/or cannabis [2]. Given that legalization of recreational cannabis across states since the time of this survey, the number of adolescents exposed to this drug will likely continue to increase through both primary and second-hand exposure. Importantly, studies in humans examining co-use of these drugs have found that individuals who reported smoking both cannabis and tobacco cigarettes consumed more cigarettes than those using tobacco alone [3]. Furthermore, the practice of mulling (combining tobacco with cannabis to smoke as a joint) has been reported as frequently occurring in adolescent users, with high incidence (up to 90%) among daily cigarette smokers in some populations [4, 5]. Interestingly, chronic male cannabis users show decreased activation of the caudate nucleus in relation to reward anticipation as compared to nicotine users and non-smokers [6], suggesting altered function of reward-related circuitries dependent on prior drug exposure. Chronic use of cannabis during adolescence has also been linked to an elevated risk of psychosis, anxiety disorders, and depression [7]. For instance, Crane and colleagues found that symptoms of depression were positively correlated with both cannabis use and tobacco smoking frequency in male, but not female, subjects [8–10]. In contrast, Wright and colleagues report that cannabis use predicted increased depressive symptoms in both males

and females, but increased anxiety symptoms and behavioral disinhibition were only found in females [9]. Adolescent substance users have also been found to exhibit abnormalities in brain function, structure, and volume [10]. However, given the nature of human studies, it is difficult to establish a causal link between early life exposure and the development of these conditions, especially as drug co-use is not often considered and may partially explain inconsistent findings noted in prior studies.

Nicotine acts in the brain via the neuronal nicotinic acetylcholine receptors, which are ligand-gated ion channels expressed on both presynaptic and postsynaptic membranes [11, 12]. Rodent models have shown that adolescent nicotine exposure alone may lead to behavioral alterations during adulthood. For instance, in male and female rats, adolescent nicotine enhances nicotine reward and intake during adulthood [13, 14]. Nicotine during adolescence has also been shown to increase depression-associated behaviors, decrease exploratory activity, and induce deficits in context conditioning to shock-associated cues in adult rats [15–17]. However, in these studies, differences were not found with anxiety-associated behaviors, extinction of contextual conditioning, or cued fear responses [15–17]. In mice, sex dependent effects have been noted, with adolescent nicotine consumption leading to decreased anxiety-associated behaviors in adult females, but not males [18]. With regard to cannabinoids, Δ9-tetrahydrocannabinol (THC) has been classified as the main psychoactive component in cannabis and exerts its actions on cannabinoid 1 (CB1) and cannabinoid 2 (CB2) receptors in the brain and periphery. Differential patterns of expression for these receptors are found across adolescent development and between males and females, and notably CB1 receptors exhibit the highest level of expression during the developmental period of mid-adolescence [19, 20]. Following THC administration in

adolescence, adult female, but not male, rats exhibit depression-associated behaviors, but no changes in anxiety-associated or general locomotor behaviors were observed [21]. Interestingly, the depression-associated behavioral effects found in females were paralleled by significantly reduced CB1 receptor expression and activity in the amygdala, ventral tegmental area and nucleus accumbens, whereas similar changes were not found in the ventral tegmental area and nucleus accumbens of males [21]. Further, administration of WIN 55,212–2, a CB1 and CB2 specific agonist, during adolescence has similarly been shown to increase depressive-like behaviors, as well as palatable food intake, during adulthood in male rats [22, 23]. Together, these prior findings demonstrate that early life exposure to either nicotine or cannabinoid agonists alone can alter later affective and cognitive function, which introduces the possibility of potential synergistic or opposing effects under co-use conditions.

In the current studies, we sought to examine whether nicotine and cannabinoid co-exposure during mid-adolescence would result in altered affective and reward-seeking behavior during adulthood. While prior studies have examined each drug and/or behavioral measure independently, the current investigations represent the first study of a co-exposure condition, which is commonly found in human subjects, and the resulting effects on multiple cognitive and affective measures. To this end, adolescent mice were exposed to the cannabinoid receptor agonist, WIN55,212–2, and/or nicotine and then assessed for cognitive, anxiety-related and depression-related behaviors during adulthood. Drug exposure occurred during postnatal day 38–49, which corresponds to mid-adolescence in rodents or ~13–17 years of age in humans [19, 24]. Based on prior evidence of differential responses for males and females with drug-related effects and baseline receptor expression

[7, 19, 25], male and female mice were examined in a within-sex manner. Further, given that significant differences were found in behavioral measures at the moderate dose of the cannabinoid agonist, a second study was then conducted to examine whether these effects would be maintained with a lower dose of the cannabinoid agonist. Together, these studies provide evidence that adolescent drug exposure alters affective and reward-related behaviors during adulthood in a sex- and drug-dependent manner.

METHODS

Animals

Male and female wildtype C57BL/6J mice were derived from breeders in our laboratory animal facilities. Mice were maintained in an environmentally controlled vivarium on a 12 h reversed light/dark cycle. Food and water were provided ad libitum until behavioral training commenced. During food training, subjects were mildly food restricted to 85–90% of their free-feeding bodyweight, and water was provided ad libitum. Following food training and the lever reversal task, food and water were again provided ad libitum for at least 5 days prior to subsequent behavioral assessments. All experiments were conducted in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee at the University of California, Irvine.

Drugs

The cannabinoid receptor agonist WIN55,212–2 mesylate (Tocris/Bio-Techne Corp, Minneapolis, ME, USA) was dissolved in vehicle containing 1% DMSO, 1% Tween-80, and 98% saline (sterile 0.9% NaCl). The doses of WIN55,212–2 administered were 2 or 0.2

mg/kg intraperitoneally (i.p.). The moderate dose of WIN (2 mg/kg) was selected based on prior studies demonstrating altered neural function with adolescent exposure in mice and rats [26, 27], and the low dose of WIN (0.2 mg/kg) was selected since this amount of drug has been shown to sustain daily reinforcing self-administration behavior in adolescent rats (~16 infusions/day at 0.0125 mg/kg/infusion = ~0.2 mg/kg per day) [28]. (-)-Nicotine hydrogen tartrate salt (MP Biomedicals, Santa Ana, CA, USA; 0215355491) was dissolved in 0.9% sterile saline and adjusted to pH 7.4. Nicotine was administered at a dose of 0.36 mg/kg, subcutaneous (s.c.) (free-base form); this dose is within the rewarding range of the dose response function that also elicits a behavioral response in adolescent C57BL/6J mice [29, 30]. Peripheral injections were administered at a volume of 10 mL/kg.

Adolescent injection schedule

Beginning on postnatal day (PND) 38, the first groups of male and female mice were randomly subdivided into four experimental groups: (1) Control (saline s.c., vehicle i.p.), (2) NIC (0.36 mg/kg nicotine s.c., vehicle i.p.), (3) WIN (saline s.c., 2 mg/kg WIN i.p.), and (4) NIC/WIN (0.36 mg/kg nicotine s.c., 2 mg/kg WIN i.p.). Saline and vehicle were the solutions used to dissolve nicotine and WIN, respectively. Mice received once daily injections for 12 consecutive days from PND 38 to PND 49. The daily injection schedule was selected to model an experimental pattern of adolescent exposure. Body weight was recorded prior to each injection. The second study included mice treated as above, but they were subdivided into the following experimental groups: 1) Control (saline s.c., vehicle i.p.), (2) LdWIN (saline s.c., low dose (0.2 mg/kg) WIN i.p.), and (3) NIC/LdWIN (0.36 mg/kg nicotine s.c., 0.2 mg/kg WIN

i.p.). For both studies, subjects were tested in multiple smaller cohorts to enhance rigor and reproducibility of the findings.

Operant food training

On PND 70, subjects were mildly food restricted and trained to press a lever in an operant chamber (Med Associates, Fairfax, VT, USA) for food pellets (20 mg; TestDiet) under a fixed-ratio 5, time out 20 s (FR5TO20s) schedule of reinforcement. Each session was performed using 2 retractable levers (1 active, 1 inactive). Completion of the response criteria on the active lever resulted in the delivery of a food pellet. Responses on the inactive lever were recorded but had no scheduled consequences. Once stable responding was achieved (criteria >30 pellets per session across consecutive 3 sessions), the lever assignment was switched to examine cognitive flexibility. In the reversal task, the previous inactive lever became active, in that food pellets were earned in accordance with the established FR5TO20s schedule. In contrast, the previously active lever became inactive, in which responses were recorded but without scheduled consequence. All behavioral responses were automatically recorded by MedAssociates software.

Open field locomotor test

The open-field chamber was composed of Plexiglas (35 cm L × 35 cm W × 31 cm H). After a 5-minute habituation period, subjects were scored in the open-field apparatus for a 15-minute test to assess locomotor activity. Activity was recorded with a video camera and scored by two experimenters blinded to the group condition with ANY-Maze Software (Stoelting Co., Wood Dale, IL, USA).

Elevated plus maze

The elevated plus maze (EPM) was composed of 4 opaque runways 5 cm wide and 35 cm in length, which were elevated 40 cm from the floor. Two opposing closed runways had opaque walls 15 cm in height, whereas the other two opposing sides did not contain walls (open arms). Subjects were placed in the center portion of the elevated plus maze and behavior was recorded for 5 min with a video camera. Behavior was scored by two blinded experimenters with ANY-maze software.

Sucrose consumption

Subjects were habituated to sucrose pellet consumption for 2 days prior to sucrose testing, during which time 60 mg of sucrose pellets (raspberry flavored; TestDiet, St. Louis, MO, USA) was provided for each subject in the home cage. On the third day, subjects were individually examined in home cage conditions, but were single housed and provided 200 mg of total sucrose pellets in a dish. All subjects were maintained under ad libitum full food conditions, and thus were not food restricted during testing. Sucrose eaten was recorded at specified intervals (5, 10, 15, 20, 30, 40, 50, 60 min) by experimenters blinded to the group condition. At the end of each session, experimenters examined the cage for breakage or disintegration of sucrose pellets; this occurred on only a few occasions and in these instances, the remnant amount was calculated and included in the final mg amount of sucrose remaining. Mice were required to consume at least one 20mg sucrose pellet within the first 30-min time period for inclusion in the study.

Chow food consumption

Subjects were examined for their daily intake of mouse chow. Mice were restricted to daily feeding sessions of 6 hr periods. During these sessions, subjects were individually housed and provided full access to consume 6–8 grams of standard chow (LabDiet 5P76, TestDiet), and water was provided in the feed cages ad libitum. Food was weighed prior to and after each session. After 3 days of habituation to the feeding protocol, data were collected on the fourth day and analyzed across groups.

Forced swim test

A cylindrical tank (22.5 cm diameter x 26 cm height) was filled with room temperature (23–25°C) water at a level of 15cm from the bottom of the tank. For testing, each subject was held by the tail, and slowly placed in the water. Mice were videotaped for the 5 min swim test duration. Data were quantified by experimenters blinded to the group assignment. Analysis of distance traveled was assessed with AnyMaze software, and the quantity of immobility bouts was hand scored by two separate experimenters to ensure accurate assessments.

Statistical analyses

Given that these studies sought to investigate the effects of drug exposure relative to the control condition within each sex, statistical comparisons were performed separately for males and females based on this a priori hypothesis. Data were analyzed by a t-test, one-way or two-way ANOVA with Prism 7 software (GraphPad, La Jolla, CA, USA), as appropriate. Data obtain across sessions was analyzed with a repeated measures two-way ANOVA. Significant main or interaction effects were followed by Bonferroni post-hoc comparison with correction for multiple comparisons. The criterion for significance was set at $\alpha = 0.05$.

RESULTS

Experiment 1: Nicotine and Moderate Dose of WIN

Body weight during adolescent injections

In an initial cohort, we assessed whether drug condition would affect change in body weight during the duration of the drug injections from postnatal day (PND) 38 (day 1 injection) to PND 49 (day 12 injection) (Fig 1.1). Change in body weight was also compared to adulthood at PND70, prior to the commencement of behavioral assessments. Groups did not differ in body weight at PND 38 following random group assignment. For males, group differences were not found when comparing the change in body weight from PND 38 to PND 49 (Fig 1.1a) (One-way ANOVA, $F(3,20) = 0.91$, $p = 0.455$) or to PND 70 (Fig 1.1c) (One-way ANOVA, $F(3,20) = 1.536$, $p = 0.236$). In contrast, female subjects exhibited a statistically significant difference in body weight change at PND 49 (Fig 1.1b) (One-way ANOVA, $F(3,29) = 4.27$, $p = 0.013$), with post-hoc tests revealing a decrease in body weight for the WIN group compared to the control ($p<0.001$). However, these effects were ameliorated during the post-injection time period, as no significant differences among the groups were found at PND 70 (Fig 1.1d) (One-way ANOVA, $F(3,29) = 0.101$, $p = 0.959$).

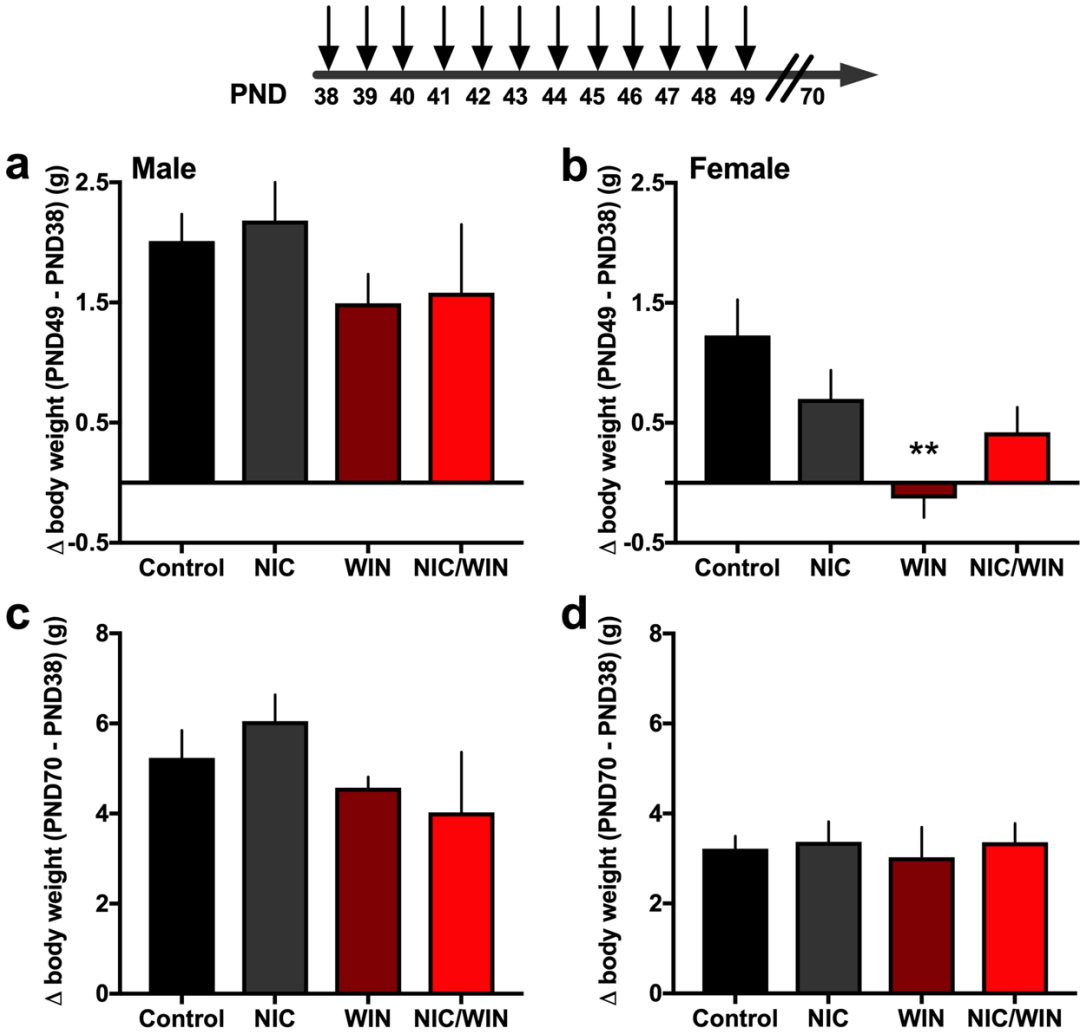

Fig 1.1 Adolescent drug exposure paradigm and change in body weight with nicotine and/or a moderate dose of the cannabinoid agonist, WIN.
(a) Male mice (n = 5-8/group) were examined for their change in body weight from the first injection on PND 38 to the final day of the injection series on PND 49. Statistically significant group differences were not found. (b) Female mice (n = 6-12/group) were examined for their change in body weight from PND38 to PND 49, and a significant difference was found with the female WIN-treated group exhibiting a decrease as compared to the control, and this effect was reversed under the co-exposure condition. **p<0.001 (c) During adulthood at PND 70, body weight differences were again not found based on adolescent drug exposure in males. (d) Females from all groups exhibited a similar increase in body weight when assessed on PND 70. Control: saline and vehicle injection group; NIC: nicotine and vehicle injection group; WIN: saline and 2 mg/kg WIN-55,212–2 injection group; NIC/WIN: nicotine and 2 mg/kg WIN-55,212,2 injection group. Data represent mean values ± SEM.
https://doi.org/10.1371/journal.pone.0211346.g001

Operant learning

Groups were examined for their ability to learn an operant task to respond for food reward. All exposure groups exhibited similar learning curves in earning food pellets for both males (Fig 1.2a) (Repeated measures two-way ANOVA, Group: $F(3,33) = 0.26$, $p = 0.853$; Session: $F(6,198) = 68.02$, $p<0.0001$; Interaction: $F(18,198) = 0.78$, $p = 0.721$) and females (Fig 1.2b) (Repeated measures two-way ANOVA, Group: $F(3,30) = 0.29$, $p = 0.835$; Session: $F(6,180) = 79.7$, $p<0.0001$; Interaction: $F(18,180) = 0.73$, $p = 0.783$). When comparing active and inactive lever pressing, all groups exhibited a clear dissociation between the active and inactive lever consistent with learned behavior in the operant task. For males, significant main and interaction effects were found (Fig 1.2c) (Repeated measures two-way ANOVA, Group: $F(7,66) = 71.86$, $p<0.0001$; Session: $F(6,396) = 93.39$, $p<0.0001$; Interaction: $F(42,396) = 13.62$, $p<0.0001$). For females, similar differences were also found (Fig 1.2d) (Repeated measures two-way ANOVA, Group: $F(7,60) = 105.2$, $p<0.0001$; Session: $F(6,360) = 128.1$, $p<0.0001$; Interaction: $F(42,360) = 20.51$, $p<0.0001$). For both males and females, post-hoc tests revealed significant differences between the number of active and inactive lever presses for all groups from sessions 3–7, but the groups did not differ from one another when comparing responding among drug conditions on each lever.

After establishing consistent responding on the active lever, cognitive flexibility was examined in the reversal task. Subjects were required to switch their lever pressing behavior, as the active and inactive lever assignments were reversed. Interestingly, the groups did not differ during the reversal session for their total number of rewards earned (males, one-way ANOVA, $F(3,33) = 1.86$, $p = 0.156$; females, one-way ANOVA, $F(3,30) = 0.32$, $p = 0.814$) or for the within-session active to inactive lever pressing ratio for both males (Fig

1.2e) (One-way ANOVA, $F(3,33) = 1.88$, $p = 0.153$) and females (Fig 1.2f) (One-way ANOVA, $F(3,30) = 0.92$, $p = 0.443$). Groups also did not differ in the latency to respond on the active lever for males (One-way ANOVA, $F(3,32) = 0.35$, $p = 0.787$) and females (One-way ANOVA, $F(3,30) = 0.25$, $p = 0.861$). Next, we obtained a reversal index, which was derived by the equation: ((number of active lever presses during the reversal session)/(number of active lever presses during the baseline session prior to reversal))*100. Surprisingly, the male WIN and NIC/WIN groups exhibited a higher reversal index, indicating greater cognitive flexibility (Fig 1.2g) (One-way ANOVA, $F(3,33) = 5.19$, $p = 0.004$). In contrast, differences in the reversal index were not found among the female groups (Fig 1.2h) (One-way ANOVA, $F(3,30) = 0.41$, $p = 0.748$).

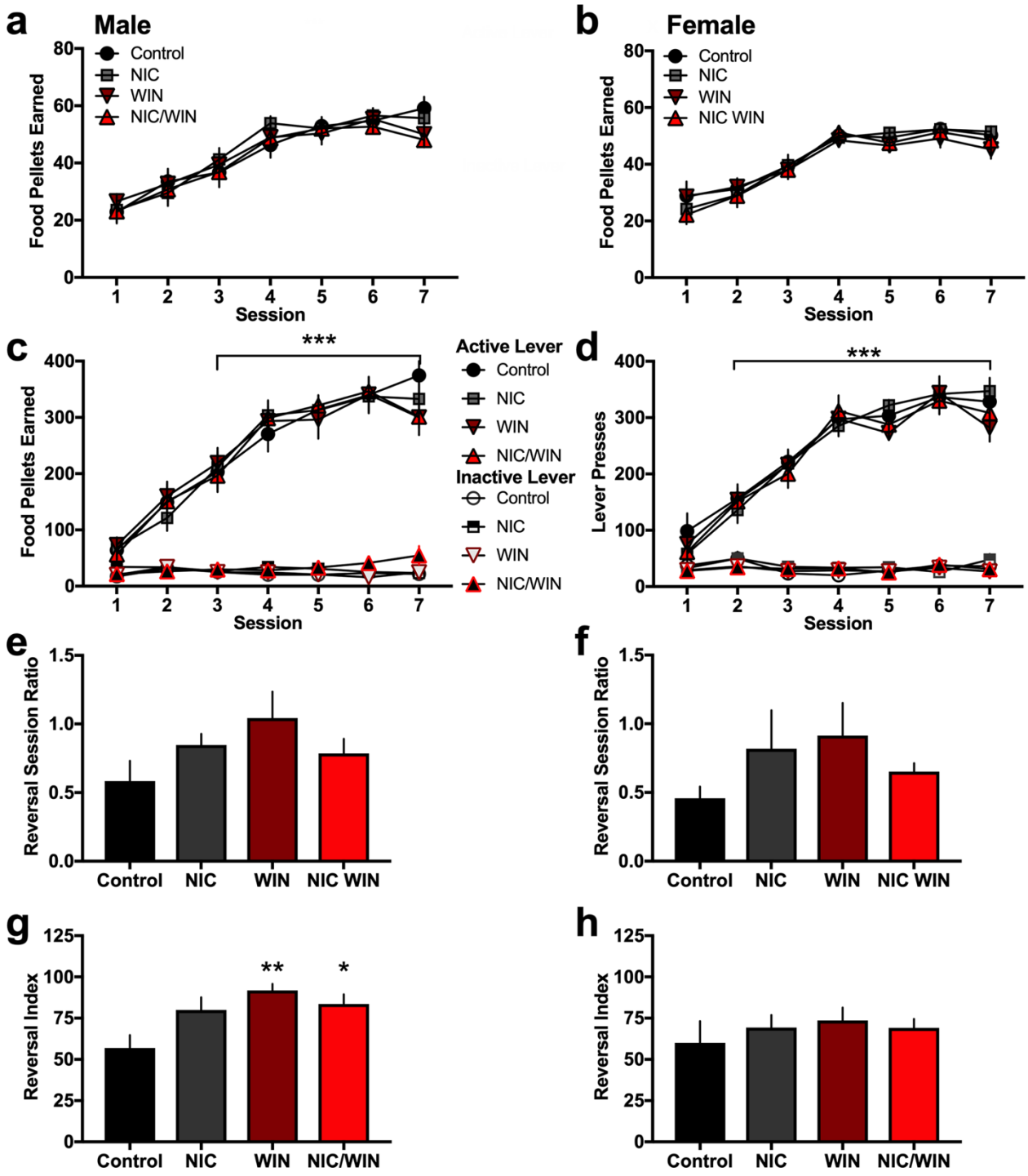

Fig 1.2 Operant learning and cognitive flexibility following adolescent exposure to nicotine and/or a moderate dose of the cannabinoid agonist in adult mice.
(a) Male mice (n = 9-10/group) were examined for their ability to learn an operant task to obtain food reward. Groups did not differ in their number of food pellets earned across sessions. (b) Female mice (n = 7-9/group) were examined with operant food training, and differences were not found among groups in the number of food pellets earned across sessions. (c) The number of active and inactive lever presses was examined across sessions

for all groups. Significant main and interaction effects were found with all groups exhibiting statistically significant preference for the active lever versus the inactive lever for sessions 3–7. ***p<0.0001 (d) Female mice also exhibited significant main and interaction effects for all groups when comparing number of active to number of inactive lever presses for sessions 2–7. ***p<0.0001 (e-h) In the cognitive flexibility assessment, mice were required to reverse their lever pressing behavior for the active and inactive lever. During the reversal session, the ratio of the number of active to inactive lever presses was derived (number active/number inactive). The reversal index was also calculated as a comparison to the baseline day of responding prior to the lever switch ((number active reversal session/number active baseline session)*100). (e) Male mice did not exhibit any group differences in the active:inactive ratio. (f) Female mice also did not exhibit any group differences in the active:inactive ratio. (g) For the reversal index, the male WIN and NIC/WIN groups exhibited increased lever pressing behavior on the reversal session, as evidenced by the higher reversal index for these groups compared to the control. **p<0.01 (h) Female mice did not exhibit any group differences in the reversal index. Data represent mean values ± SEM. https://doi.org/10.1371/journal.pone.0211346.g002

Locomotion

The open field test was utilized to assess generalized locomotion and exploratory behavior. No statistically significant differences were observed among drug conditions in distance travelled for males (Fig 1.3a) (One-way ANOVA, $F(3,35) = 1.13$, $p = 0.351$) and females (Fig 1.3b) (One-way ANOVA, $F(3,27) = 0.90$, $p = 0.456$). Further, for the duration of time spent in the center of the open field, no differences were found among groups for males (Fig 1.3c) (One-way ANOVA, $F(3,35) = 0.17$, $p = 0.918$) and females (Fig 1.3d) (One-way ANOVA, $F(3,27) = 0.71$, $p = 0.553$).

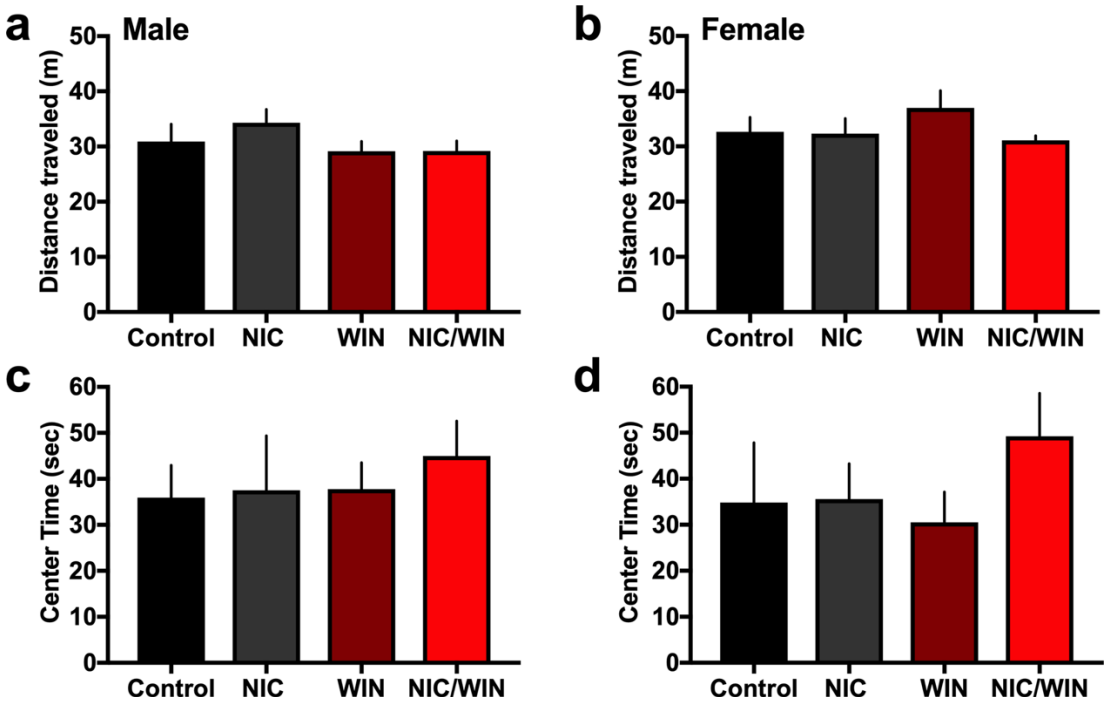

Fig 1.3 Adolescent nicotine and/or cannabinoid agonist exposure does not alter locomotor behavior during adulthood.
(a) Male mice (n = 8-12/group) did not differ in the distance travelled in the open field. (b) Female mice (n = 6-10/group) did not differ in the distance travelled in the open field. (c) Analysis of the time spent in the center of the field did not reveal any differences in male subjects. (d) Females also did not differ in the time spent in the center of the field. Data represent mean values ± SEM. https://doi.org/10.1371/journal.pone.0211346.g003

Anxiety-related assessment

As a measure for anxiety-related behavior, subjects were assessed in the elevated plus maze, in which increased time in the open arms is thought to represent an anxiolytic effect. In the males, we found a significant increase in the time spent in the open arm of the elevated plus maze for both the WIN and NIC/WIN groups as compared to the control condition (Fig 1.4a) (One-way ANOVA, $F(3,26) = 5.00$, $p = 0.007$). Interestingly, the WIN only group also exhibited an increase in the number of crosses between the arms (Fig 1.4c) (One-way ANOVA, $F(3,26) = 3.72$, $p = 0.024$), and this was likely indicative of decreased anxiety related effects and/or increased exploratory behavior, rather than an overall increase in

general locomotion given the absence of effects in the above noted open field test. Moreover, the presence of nicotine with WIN resulted in no significant difference from the control condition, and thus, the co-exposure condition counteracted the WIN-induced increase in exploratory behavior. In contrast, although the data depict a trend for the females exposed to WIN or co-exposed to nicotine and WIN spending more time in the open arms, due to individual variability and lower subject numbers, statistically significant differences were not found among groups in the open arm time (Fig 1.4b) (One-way ANOVA, $F(3,18) = 0.70$, $p = 0.565$) or number of arm crosses (Fig 1.4d) (One-way ANOVA, $F(3,18) = 0.43$, $p = 0.737$).

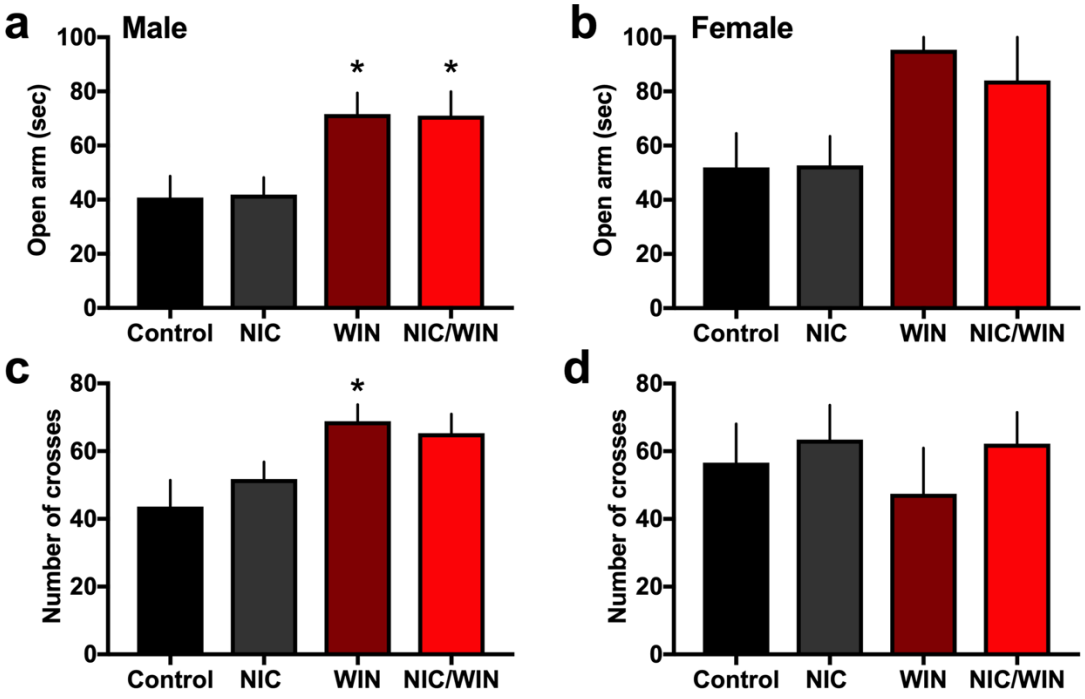

Fig 1.4 Altered anxiety-related behavior in male, but not female, adult mice following adolescent cannabinoid agonist exposure at a moderate dose.
(a) Male mice (n = 7-8/group) exhibited differential responding in the elevated plus maze dependent on adolescent drug exposure. Specifically, mice treated with the cannabinoid agonist WIN or co-treated with WIN and nicotine exhibited increased time on the open arm, indicating a decrease in anxiety-related behavior. *$p<0.05$ (b) Female mice (n = 5-7/group) did not exhibit any statistically significant differences in the elevated plus maze open arm time. (c) For the male mice, the WIN group also displayed a significant increase in the number

of crosses between arms compared to the control group, potentially indicative of increased exploratory behavior, an effect which was decreased with NIC/WIN co-exposure. *p<0.05 (d) The number of arm crosses did not differ significantly between the groups for the female mice. Data represent mean values ± SEM.
https://doi.org/10.1371/journal.pone.0211346.g004

Sucrose and food consumption

Mice were examined for their consummatory behavior of natural reward with 1hr access to sucrose pellets. Statistically significant main and interaction effects were found for the amount of sucrose consumed across groups in males (Fig 1.5a) (Two-way ANOVA, Group: $F(3,22) = 3.71$, $p = 0.027$; Time: $F(7,154) = 67.54$, $p<0.0001$; Interaction: $F(21,154) = 1.85$, $p = 0.018$). Post-hoc analysis revealed a significant increase in the NIC/WIN group compared to the control group at the 40-, 50-, and 60-min time points ($p<0.01$, $p<0.001$, $p<0.001$, respectively), and a significant increase for the WIN group compared to the control group at the 60 min time point ($p<0.05$). Female subjects also exhibited significant group differences (Fig 1.5b) (Two-way ANOVA, Group: $F(3,29) = 2.16$, $p = 0.115$; Time: $F(7,203) = 111.5$, $p<0.0001$; Interaction: $F(21,203) = 3.35$, $p<0.001$), with the post-hoc analysis revealing a decrease in consumption for the WIN group relative to the control group at time points 40, 50 and 60 min ($p<0.01$, $p<0.01$, $p<0.0001$, respectively). Differences were not found among groups for the initial latency to consume a sucrose pellet (males, one-way ANOVA, $F(3,23) = 0.11$, $p = 0.99$; females, one-way ANOVA, $F(3,29) = 0.43$, $p = 0.733$). To ensure that the sucrose consumption was not secondary to general food intake among the groups, subjects were also assessed for their daily consumption of mouse chow. Male groups did not differ in the amount of food consumed (one-way ANOVA, $F(3,30) = 0.31$, $p = 0.82$) (Fig 1.5c), nor did the females groups (one-way ANOVA, $F(3,28) = 2.41$, $p = 0.09$) (Fig 1.5d).

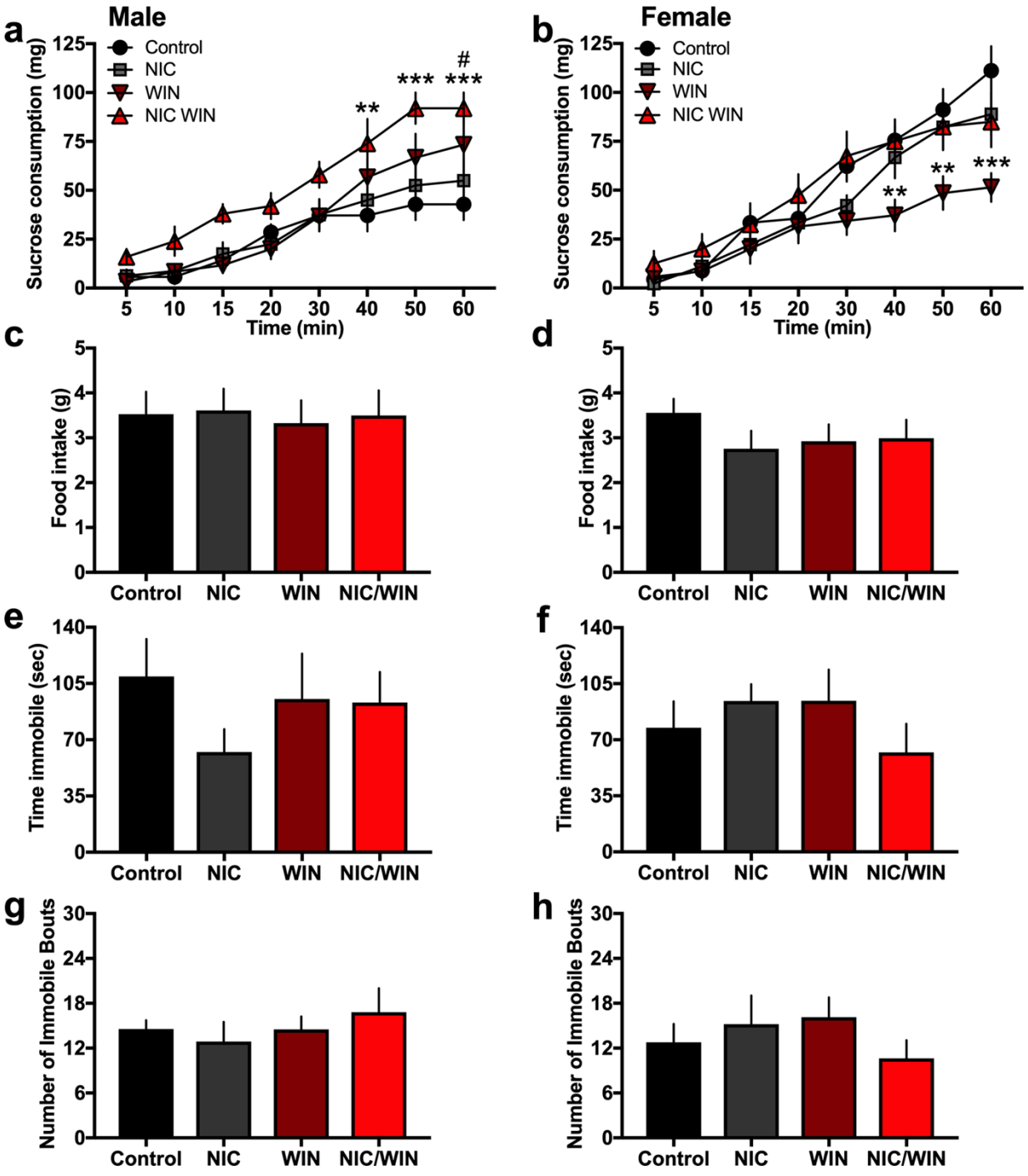

Fig 1.5 Within sex-specific effects in natural reward consumption, but not other depression-associated behaviors, in adult mice following adolescent exposure to nicotine and/or a moderate dose of WIN.

(a) Male subjects (n = 5-8/group) were examined for cumulative sucrose consumption during a 1 hr test. The NIC/WIN mice exhibited a significant increase in sucrose consumption at the 40, 50 and 60 min time points, as compared to the control group. **p<0.01, ***p<0.001 Further, the WIN group consumed greater sucrose than the control group at the 60 min time

point. #p<0.05 (b) In contrast, female mice (n = 7-9/group) exhibited a differential effect, with the WIN treated group consuming less sucrose pellets than the control group at the 40, 50 and 60 min time points. **p<0.01, ***p<0.001 (c-d) Since sucrose consumption could be secondary to generalized food intake among groups, subjects were examined for standard chow intake during a restricted 6hr daily feeding period. Male groups did not differ in chow food intake (c), nor did the female groups (d). (e-h) To examine whether the sucrose consumption findings were consistent with other measures of depression-associated behaviors, the forced swim test was employed. Groups did not differ in the time immobile for both males (e) and females (f). Similarly, groups did not differ in the number of immobile bouts for both males (g) and females (h). Data represent mean values ± SEM.
https://doi.org/10.1371/journal.pone.0211346.g005

Depression-associated behavior

To further determine whether the differences in sucrose consumption were due to reward related effects, as predicted, or secondary to an anhedonia/depression-associated state, we next examined swim behavior in the forced swim test. In this assessment, we found no significant differences among groups in the time immobile or number of immobile bouts for both males (Fig 1.5e and 1.5g, respectively) (Time immobile: One-way ANOVA, $F(3,22) = 1.01$, $p = 0.409$; Immobile bouts: One-way ANOVA, $F(3,22) = 0.472$, $p = 0.705$) and females (Fig 1.5f and 1.5h, respectively) (Time immobile: One-way ANOVA, $F(3,29) = 0.91$, $p = 0.450$; Immobile bouts: One-way ANOVA, $F(3,29) = 0.66$, $p = 0.57$).

Experiment 2: Nicotine and Low Dose of WIN

Body weight during adolescent injections

Similar to above, we first assessed whether drug administration would alter body weight during the duration of the drug injections from postnatal day (PND) 38 (day 1 injection) to PND 49 (day 12 injection) or during adulthood at PND70 (Fig 1.6). For males, group differences were not found when comparing the change in body weight from PND 38 to PND 49 (Fig 1.6a) (One-way ANOVA, $F(2,37) = 0.60$, $p = 0.555$) or to PND 70 (Fig 1.6c) (One-way ANOVA, $F(2, 37) = 0.89$, $p = 0.419$). Female subjects also did not exhibit differences in body weight change at PND 49 (Fig 1.6b) (One-way ANOVA, $F(2,42) = 0.83$, $p = 0.444$) or at PND 70 (Fig 1.6d) (One-way ANOVA, $F(2,42) = 1.37$, $p = 0.265$).

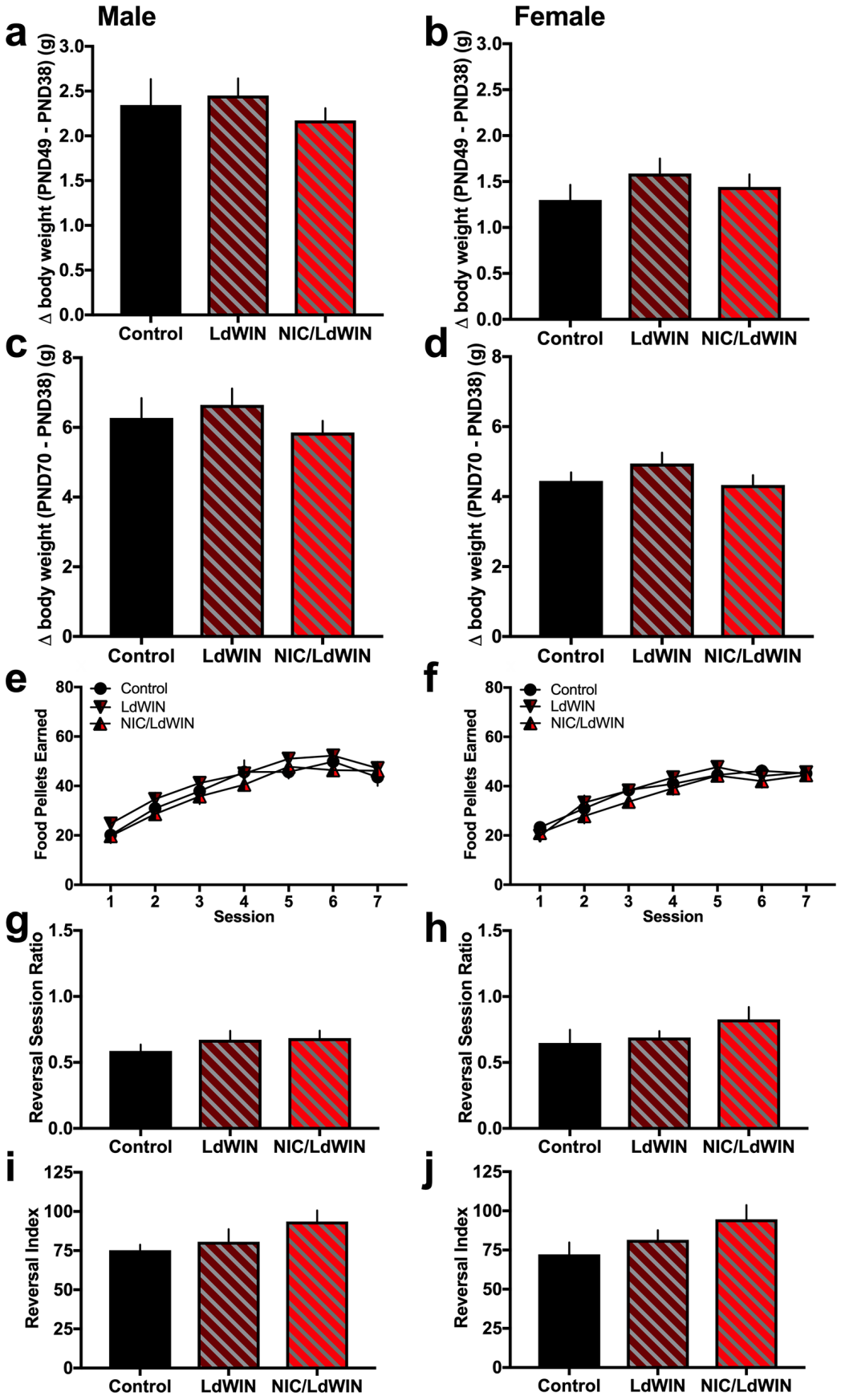
Male
Female
a
b
c
d
e
f
g
h
i
j
Δ body weight (PND49 - PND38) (g)
Δ body weight (PND70 - PND38) (g)
Control
LdWIN
NIC/LdWIN
Food Pellets Earned
Session
Reversal Session Ratio
Reversal Index

Fig 1.6 Body weight change, operant learning and cognitive flexibility following low dose exposure to the cannabinoid agonist, with or without nicotine.
(a) Male mice (n = 9-16/group) were examined for their change in body weight from the first injection on PND 38 to the final day of the injection series on PND 49. Statistically significant group differences were not found. (b) Female mice (n = 13-17/group) were examined for their change in body weight from PND38 to PND 49, and no significant differences were found. (c-d) During adulthood at PND 70, differences in body weight were again not found across adolescent drug exposure conditions for either males (c) or females (d). (e) Male mice (n = 14-16/group) across groups did not differ in their ability to learn an operant task to obtain food reward. (f) Female mice (n = 14-16/group) also did not differ in their operant responding for food reward. (g-j) In the cognitive flexibility assessment, mice were next required to reverse their lever pressing behavior for the active and inactive lever. During the reversal session, the ratio of the number of active to inactive lever presses was derived; differences among groups were not found for both males (g) and females (h). The reversal index was also calculated as a comparison to the baseline day of responding prior to the lever switch. (i) Male mice did not exhibit any group differences and equally switched their lever pressing behavior to the active lever. (j) Female mice also exhibited similar indices of cognitive flexibility in switching their behavior to respond on the reassigned active lever. Control: saline and vehicle injection group; LdWIN: saline and 0.2 mg/kg WIN-55,212–2 injection group; NIC/LdWIN: nicotine and 0.2 mg/kg WIN-55,212,2 injection group. Data represent mean values ± SEM. https://doi.org/10.1371/journal.pone.0211346.g006

Operant learning

Groups were next examined for their ability to learn an operant task to respond for food reward. All exposure groups exhibited similar learning curves in earning food pellets for both males (Fig 1.6e) (Repeated measures two-way ANOVA, Group: $F(2,41) = 1.27$, $p = 0.30$; Session: $F(6,246) = 84.69$, $p<0.0001$; Interaction: $F(12,246) = 0.68$, $p = 0.77$) and females (Fig 1.6f) (Repeated measures two-way ANOVA, Group: $F(2,43) = 0.79$, $p = 0.460$; Session: $F(6,258) = 86.37$, $p<0.0001$; Interaction: $F(12,258) = 0.84$, $p = 0.607$). After establishing consistent responding on the active lever, cognitive flexibility was examined in the reversal task. Subjects were required to switch their lever pressing behavior, as the active and inactive lever assignments were reversed. Interestingly, the groups did not differ during the reversal session for the within-session active to inactive lever pressing ratio for both males (Fig 1.6g) (One-way ANOVA, $F(2,33) = 0.56$, $p = 0.576$) and females (Fig 1.6h)

(One-way ANOVA, $F(2,38) = 1.29$, $p = 0.288$). Next, we obtained the reversal index as described above, and no significant differences were found for males (Fig 1.6i) (One-way ANOVA, $F(2,33) = 1.56$, $p = 0.225$) and females (Fig 1.6j) (One-way ANOVA, $F(2,38) = 1.97$, $p = 0.154$).

Locomotion and anxiety-related behaviors

The open field test was utilized to assess generalized locomotion and exploratory behavior in the low dose WIN groups. No statistically significant differences were observed among drug conditions for distance travelled in males (Fig 1.7a) (One-way ANOVA, $F(2,29) = 2.50$, $p = 0.099$) and females (Fig 1.7b) (One-way ANOVA, $F(2,33) = 0.82$, $p = 0.451$). Further, for the duration of time spent in the center of the open field, no differences were found among groups in males (One-way ANOVA, $F(2,29) = 0.05$, $p = 0.946$) and females (One-way ANOVA, $F(2,33) = 0.24$, $p = 0.789$). Subjects were then assessed in the elevated plus maze to examine anxiety-related and exploratory behaviors. In the males, significant differences among the groups were not found in the time spent in the open arm of the elevated plus maze (Fig 1.7c) (One-way ANOVA, $F(2,28) = 0.96$, $p = 0.395$). Similarly, differences among groups were not found for females in the open arm time (Fig 1.7d) (One-way ANOVA, $F(2, 32) = 1.36$, $p = 0.271$). With regard to the number of crosses in the elevated plus maze, differences were not present among the groups for males (Fig 1.7e) (One-way ANOVA, $F(2,28) = 0.09$, $p = 0.914$) and females (Fig 1.7f) (One-way ANOVA, $F(2, 32) = 0.52$, $p = 0.598$).

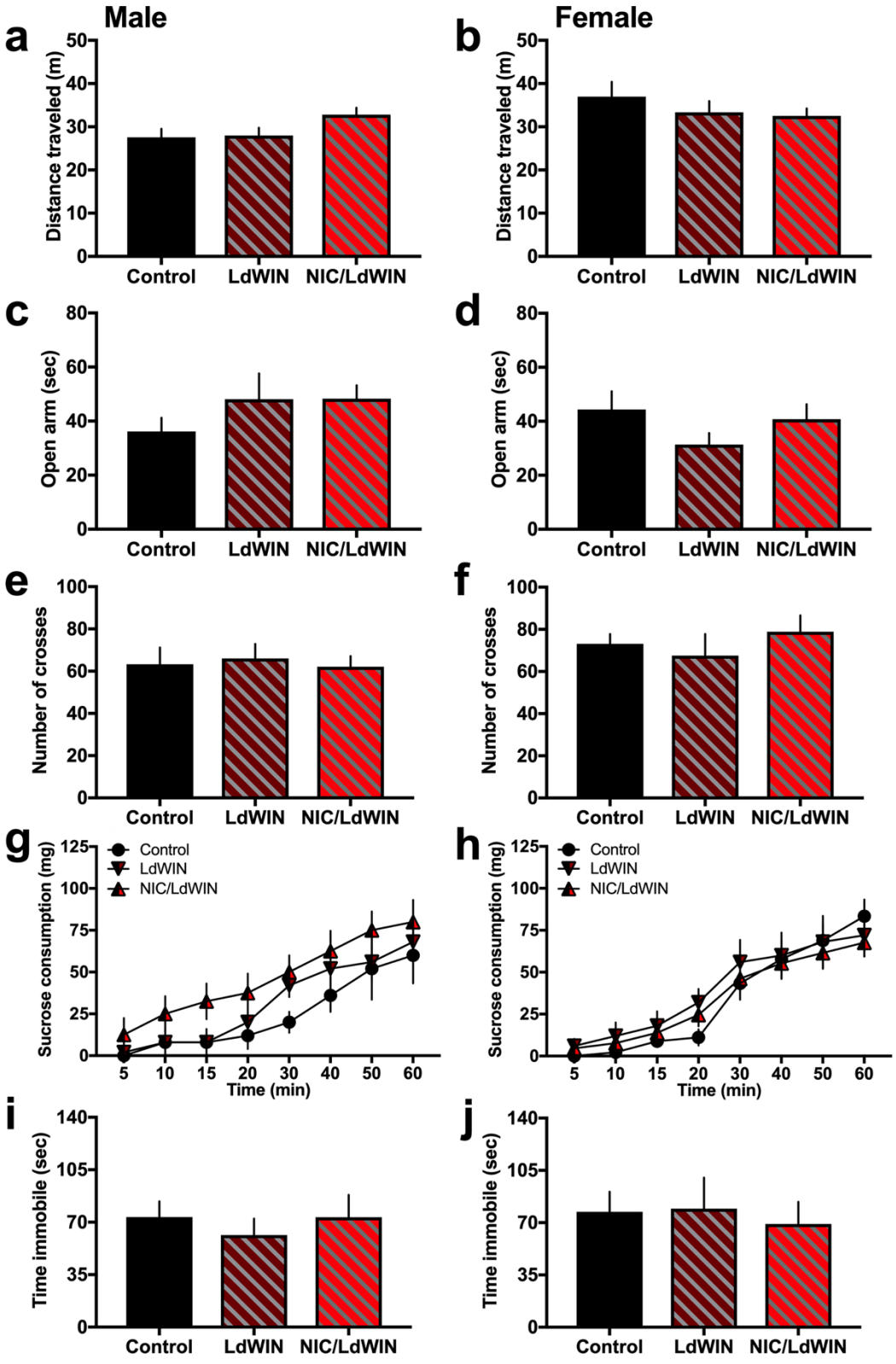

Fig 1.7 Adolescent low dose cannabinoid agonist exposure with or without nicotine does not alter locomotor or affective-associated behaviors during adulthood.

(a) Male mice (n = 10-12/group) did not differ in the distance travelled in the open field. (b) Female mice (n = 11-13/group) did not differ in the distance travelled in the open field. (c-f) To assess anxiety-associated behaviors, mice were then tested in the elevated plus maze. Statistically significant differences were not found among the groups in the time spent on the open arms for males (c) and females (d). Differences were also not found in the number of arm crosses for males (e) and females (f). (g-j) Reward related and depression-associated behaviors were then assessed across groups; differences were not found for sucrose consumption in males (g) and females (h), nor were differences found in the forced swim test for males (i) and females (j). Data represent mean values ± SEM.
https://doi.org/10.1371/journal.pone.0211346.g007

Reward and depression-associated behaviors

Groups exposed to the low dose of WIN were examined for their consummatory behavior of natural reward with 1hr access to sucrose pellets. Treatment groups did not differ for sucrose consumption in both males (Fig 1.7g) (Two-way ANOVA, Group: $F(2,20) = 1.89$, $p = 0.182$; Time: $F(7,140) = 39.67$, $p<0.0001$; Interaction: $F(14,140) = 0.49$, $p = 0.933$) and females (Fig 1.7h) (Two-way ANOVA, Group: $F(2,29) = 0.24$, $p = 0.79$; Time: $F(7,203) = 67.32$, $p<0.0001$; Interaction: $F(14,203) = 0.79$, $p = 0.677$). Finally, we examined for depression-associated behavior in the forced swim test, but no statistically significant differences were found in the time immobile for both males (Fig 1.7i) (One-way ANOVA, $F(2,25) = 0.33$, $p = 0.721$) and females (Fig 1.7j) (One-way ANOVA, $F(2,30) = 0.11$, $p = 0.893$).

DISCUSSION

Given the growing incidence of nicotine and cannabis experimentation during adolescence, we sought to examine whether such exposure would lead to altered behavioral effects during adulthood. In these studies, we found that male adolescent exposure to a moderate dose of the cannabinoid receptor agonist, WIN55,212–2 (WIN), led to increased cognitive flexibility in a learning reversal task, decreased anxiety-associated behaviors, and increased natural reward consumption, but no differences in general locomotor or depression-related behavior. Interestingly, the co-exposure condition of both nicotine and the moderate dose of WIN led to similar behavioral profiles as WIN alone in these measures, suggesting that a potentiative or additive effect was not present for these behaviors. However, with regard to the number of lane crosses in the elevated plus maze, the nicotine and WIN co-exposure condition appeared to exert a counteractive effect on the WIN-induced increase in exploratory behavior at the moderate dose, suggesting an opposing effect with adolescent exposure to both drugs. With regard to females, the moderate dose of WIN induced a lower body weight during the adolescent period, but co-exposure with nicotine appeared to exert an opposing effect that resulted in no difference from the control condition. However, these effects of WIN on body weight were transitory, as the difference in females did not persist into adulthood. For the behavioral assessments, female subjects were overall more resistant to the long-term effects of adolescent drug exposure. Group differences were only found in the sucrose consumption test, in which the moderate dose WIN females exhibited decreased natural reward consumption compared to the control females. However, differences from the control were not found with the female nicotine and WIN co-exposure condition for sucrose consumption, suggesting that the presence of

nicotine ameliorated the actions of WIN on reward circuitry during the adolescent period. In contrast, adolescent exposure to a low dose of WIN had no effect on physiological or behavioral measures, either alone or in the presence of nicotine, for both males and females. Taken together, these findings demonstrate that while adolescent cannabinoid agonist exposure at a moderate dose exerts variable effects on both physiological and behavioral measures in males and females, co-administration of nicotine surprisingly counteracted some of these effects by normalizing to control levels.

While prior studies have examined the effects of adolescent exposure of either nicotine or WIN alone on later behaviors, the current findings represent the first examination of the effects of co-exposure during mid-adolescence and subsequent long-term effects on adult behavior. This age range was selected based on the correlation to human adolescence with higher levels of experimentation and more recurrent patterns of drug consumption than that found in younger individuals. With regard to nicotine alone, opposing effects have been found in male Sprague-Dawley rats with increased depression-associated behaviors, but no difference in anxiety-associated behaviors, during adulthood [15]. However, these behavioral differences were only found at higher nicotine doses approximately twice that administered in the current study. Chronic exposure approaches with a minipump or nicotine patch at higher doses (≥5 mg/kg/day) have also demonstrated decreased exploratory activity, decreased food consumption under anxiety-related conditions, and deficits in contextual condition to shock-associated cues in Sprague-Dawley rats [16, 17]. In mice, adolescent exposure to high dose minipump (12 mg/kg/day) has also been shown to disrupt contextual fear condition, but not cued fear conditioning [31]. However, since studies have shown that of those adolescents age 12–17 who smoke, the

majority smoke one or less than one cigarette per day (50.1%)[32], the current studies focused on a rewarding dose with once daily exposure as an investigative goal. Thus, the lack of difference in the behavioral measures with nicotine exposure in the current studies may be attributed to this relatively lower dose administered. Along these lines, it should be noted that this dose was selected based on the rewarding effects of doses in this range, as assessed with the brain reward threshold measure [29], and behavioral effects elicited in adolescent mice [30], and thus, the current results have particular relevance to experimental patterns of drug consumption found in youth.

With adolescent cannabinoid agonist exposure, findings derived from prior rat studies have been somewhat variable. In one study, adolescent male and female rats treated with the cannabinoid agonist, CP 55,940, exhibited overall increased time on the open-arm of the elevated plus maze, but these effects were not maintained when examining males and females independently [33], suggesting these differences may have been confounded by baseline differences between the sexes. Since CP 55,940 has high affinity for both the CB1 and CB2 receptors, as well as GPR55, the lack of differences within each sex for drug condition may also have been due to actions on alternate signaling pathways or differences in agonist actions. Interestingly, male Sprague-Dawley rats treated with WIN, the CB1 and CB2 specific agonist, during adolescence exhibited increased depressive-like behaviors in the forced swim and sucrose consumption tests [22, 23]. In our mouse studies, we did not find any differences in these measures with the low dose of WIN and opposing effects at the moderate dose of WIN, indicating that species differences in metabolism and/or genetic heritability factors likely mediate the effects of cannabinoids on adolescent neurodevelopment. Finally, adolescent WIN exposure has also been found to increase

palatable food intake and alter attribution of incentive salience for food reward in adult male Long Evans rats [23]. The increase in natural reward-related effects with adolescent exposure is consistent with our findings at the moderate WIN dose in mice, suggesting cannabinoid exposure during adolescence similarly alters brain reward pathways to enhance subsequent responsiveness to natural reward. Interestingly, Schoch and colleagues also demonstrated increased expression of the endocannabinoids anandamide and oleoylethanolamine in the nucleus accumbens only during a food restricted state with adolescent WIN exposure in rats [23]. Thus, dependent on the availability of food and level of satiety, changes in neural systems regulating reward-related behaviors may be differentially affected in the presence of cannabinoids. Along these lines, it is interesting to note that in the current study, mice were at a satiated level (not food restricted) during sucrose consumption, during which time the opposing differences were found in males and females exposed to adolescent WIN. However, during conditions of food restriction, such as during operant food training in the current study, group differences only emerged for males in the reversal task. Thus, altered endocannabinoid signaling may account for this effect during the food restricted state, whereas other mechanisms likely underlie the behavioral differences observed in the anxiety and natural reward-related measures.

Cannabinoid and nicotinic acetylcholine receptors exhibit overlapping expression within brain regions implicated in reward-related and affective behaviors, including the prefrontal cortex, ventral tegmental area, nucleus accumbens, medial habenula, interpeduncular nucleus and hippocampus [7, 34]. On the cellular level, both receptors types are expressed on presynaptic terminals and function to modulate release of various neurotransmitters. For instance, with acute administration, both drugs increase

extracellular dopamine in the nucleus accumbens and prefrontal cortex [35, 36], and adolescent cannabinoid or nicotine exposure have also been shown to affect cholinergic, serotonergic and noradrenergic signaling mechanisms [22, 31, 37]. Thus, in consideration of the effects of nicotine and cannabinoids on several neurotransmitter systems and the behavioral findings from the current studies, future studies will need to dissect the differential impact of single or co-drug exposure during adolescence on neural signaling mechanisms.

In conclusion, activation of cannabinoid receptors with or without nicotine led to differential sex-specific effects on anxiety- and reward-related behaviors during adulthood. Together, these studies provide evidence that adolescent exposure to drugs of abuse may lead to alterations in affective and cognitive behaviors during adulthood. These data support the conclusion that consumption of cannabis by youth may alter later cognitive function, and thus, policy approaches should be considered to discourage and/or restrict substance use by this vulnerable population.

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Chapter 2: Adolescent Cannabinoid and Nicotine Exposure Differentially Alters Adult Nicotine Self-Administration in Males and Female

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ABSTRACT

During adolescence, exposure to nicotine or cannabis independently induces effects on neuromaturation and later cognitive function. However, the potential effect of both drugs under co-use conditions has become of increasing concern given the prevalence of e-cigarettes, legalization of cannabis, and availability of synthetic "spice" cannabinoid agonists. The current studies investigated the effects of exposure to a cannabinoid receptor agonist (WIN55,212-2) and/or nicotine over a discrete time period in mid-adolescence on later intravenous nicotine self-administration in adult male and female mice. We further examined whether cannabinoid agonist administration in adulthood would alter nicotine reinforcement, with either acute or chronic pairing across 7 days. We found that adult males exhibited increased nicotine self-administration at a lower, rewarding nicotine dose following adolescent cannabinoid exposure, either alone or with nicotine coadministration. In contrast, adult females demonstrated an opposing effect in which adolescent cannabinoid and nicotine coexposure resulted in decreased nicotine intake compared with the nicotine only and control groups. Furthermore, after maintaining nicotine self-administration across sessions, pretreatment with a low dose of the cannabinoid agonist decreased nicotine intake in both male and female control mice, and this lowering effect was evidenced after both acute and chronic treatment. However, the cannabinoid agonist was ineffective in altering nicotine intake in mice previously exposed to nicotine, cannabinoid agonist, or both during adolescence. These data provide evidence that adolescent drug exposure can alter later nicotine reinforcement in a sex-specific manner and can further modulate the effectiveness of interventions in reducing nicotine intake during adulthood. These studies demonstrate a significant impact of nicotine, cannabinoids, or coexposure on developmental processes

during adolescence. Differential effects were observed within each sex, with opposing results found for cannabinoid exposure on nicotine intake in males and females. Intriguingly, we also evidenced resistance to the lowering effects of a cannabinoid agonist on nicotine intake in adulthood based on adolescent drug exposure. Thus, these findings have important implications for our understanding of the impact of nicotine and cannabinoids (eg, Δ9-tetrahydrocannabinol (THC) and synthetic "spice" cannabinoids) during development, with further implications for the effectiveness of therapeutic interventions based on prior drug exposure in youth.

INTRODUCTION

Nicotine dependence is among the largest preventable causes of disease and death worldwide. Further, polydrug use, including that of nicotine and cannabis, may lead to interactive effects on brain neurocircuitries [1]. Thus, this study represents the first to begin deciphering the coconsumption effects of nicotine and cannabinoids during adolescent development on later dependence and/or resistance to achieving abstinence. According to a 2015 nationwide survey, 32.3% of high school students self-reported prior cigarette use, whereas 44.9% reported using vaporized nicotine products [2]. Of further concern, 38.6% of these students reported using cannabis [2]. Given that recreational cannabis use was illegal in most states at the time of this survey, the number of adolescents exposed to this drug will likely only increase through both primary use and secondhand exposure as the drug becomes more readily accessible. This is supported by the finding that 44% of 12th graders in a recent 2018 nationwide survey reported using cannabis in their lifetime [3]. Further, the practice of mulling, combining tobacco with cannabis to smoke as a joint, has been reported as frequently occurring in adolescent users, with highest incidence (up to 90%) among daily cigarette smokers in some populations [4,5]. Furthermore, individuals who reported smoking cannabis and tobacco cigarettes consumed more cigarettes than those smoking cigarettes alone [6]. Together, these findings have introduced increasing concerns regarding the interaction between the drugs and the effects of early adolescent exposure on later drug-taking behaviors.

Nicotine, the main psychoactive component in tobacco and e-cigarettes, acts in the brain on neuronal nicotinic acetylcholine receptors (nAChRs), and the psychoactive effects of cannabis have been attributed to action on the cannabinoid 1 receptor (CB1R). The CB1Rs

are also targeted by other abused drugs, such as synthetic “spice” cannabinoid agonists for which the majority belong to the aminoalkylindole class, including WIN55,212-2 [7–9]. The nAChRs and CB1Rs exhibit overlapping expression patterns within brain regions implicated in drug reinforcement and aversion, including the prefrontal cortex, ventral tegmental area, nucleus accumbens, medial habenula, interpeduncular nucleus, and hippocampus [10,11]. On the cellular level, CB1Rs and nAChRs are expressed on presynaptic axon terminals, and both function to modulate release of neurotransmitters [11,12]. Reciprocal outcomes are found in their actions and behavioral effects. Exogenous cannabinoids can modulate cholinergic neurotransmission in the brain, [13] and similarly, nicotine administration alters endogenous cannabinoid signaling [14]. Further, similar effects are found with neurotransmitter release; for instance, administration of either nicotine or the CB1R agonist, WIN55,212-2, increases extracellular dopamine in the nucleus accumbens and prefrontal cortex [15,16]. These findings provide evidence to support the notion that exogenously derived cannabinoid or cholinergic modulation of neurotransmission during adolescence may lead to various altered drug-associated behaviors along the continuum of the dependence processes.

In humans, tobacco exposure during development has been associated with increased drug use during adulthood [17,18]. However, given the nature of human studies, it is unclear as to whether the early life exposure increases vulnerability, or whether a preexisting neural state and/or environmental factors prompted consumption of the drug products. In rodents, adolescent nicotine exposure results in increased time spent in an environment associated with nicotine during adulthood, [19] suggesting an enhanced rewarding effect of nicotine following prior exposure. In an oral self-administration study, rats that drank a nicotine

solution during late adolescence into early adulthood (postnatal day [PND] 35–77) exhibited either a similar level or diminished nicotine drinking behavior in later adulthood (PND 140+) [20]. However, high variability in the amount of nicotine consumed has been found in such oral drinking paradigms, [20] potentially due to activation of nAChRs expressed in the tongue and/or postconsummatory gastrointestinal effects. In contrast, the intravenous nicotine self-administration procedure is generally accepted as having greater translational relevance to human behavior, as stable responding and titration of intake are found across doses [21,22]. A few studies have examined adolescent nicotine exposure on later nicotine self-administration in adulthood. In one study in rats, nicotine exposure during PND 25–42 did not alter later nicotine self-administration behavior during early adulthood, [23] but it should be noted that the subjects in this study were individually housed and shipped during PND 20–21 [23]; factors that could have elicited stressful conditions during the adolescent period. In contrast, another study found a decrease in the motivation to self-administer nicotine during adulthood; in this paradigm, subjects had variable access to a range of nicotine doses for self-administration, including high aversive doses, beginning at PND 34, and prior to adult testing, [24] which may have subsequently biased the resultant level pressing behavior.

Here, we sought to examine whether adolescent exposure to nicotine and/or a cannabinoid agonist would alter intravenous nicotine self-administration during adulthood in male and female mice. The current investigations focus on the coexposure condition, which is commonly found in human subjects, and the resulting effects on later nicotine intake. Adolescent mice were exposed to a moderate or low dose of the cannabinoid receptor agonist, WIN55,212-2, and/or nicotine and then were assessed for nicotine reinforcement

behaviors during adulthood. Drug exposure occurred during PND 38–49, which corresponds to mid-adolescence in rodents or ~13–17 years of age in humans [25,26]. Given the previously established differential responses for males and females with drug-related effects and baseline receptor expression across development, [11,25,27,28] male and female mice were examined in a within-sex manner. Finally, we also examined whether acute or repeated administration of the cannabinoid agonist during adulthood would alter nicotine self-administration dependent on the prior adolescent exposure condition. The goal of this study was to determine if an interaction effect would occur during adulthood, in consideration of each adolescent exposure condition. Together, these studies provide evidence that adolescent drug exposure alters nicotine reinforcement in a sex-dependent manner and prevents the dampening effects of a cannabinoid on nicotine intake during adulthood in both sexes.

METHODS

Animals

Male and female wild-type C57BL/6J mice were derived from breeders in our laboratory animal facilities; in total, 54 male and 63 female mice were examined in these studies. Mice were maintained in an environmentally controlled vivarium on a 12-hour reversed light/dark cycle. Food and water were provided ad libitum until behavioral training commenced. During food and nicotine self-administration, subjects were mildly food restricted to 85%–90% of their free-feeding body weight, and water was provided ad libitum. All experiments were conducted in strict accordance with the NIH Guide for the Care

and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at the University of California, Irvine.

Drugs

The cannabinoid receptor agonist WIN55,212-2 mesylate (Tocris/Bio-Techne Corp, Minneapolis, ME) was dissolved in vehicle containing 1% dimethyl sulfoxide, 1% Tween-80, and 98% saline (sterile 0.9% NaCl). The doses of WIN55,212-2 administered were 0.2 or 2 mg/kg, intraperitoneally (i.p.). The moderate dose of WIN (2 mg/kg) was selected based on prior studies demonstrating altered neural function with adolescent exposure in mice and rats, [29,30] and the low dose of WIN (0.2 mg/kg) was selected based on evidence from adolescent WIN self-administration in rats (~16 infusions/day at 0.0125 mg/kg/infusion = ~0.2 mg/kg/day) [31]. (−)-Nicotine hydrogen tartrate salt (MP Biomedicals, Santa Ana, CA; 0215355491) was dissolved in 0.9% sterile saline and adjusted to pH 7.4. Nicotine was administered at a dose of 0.36 mg/kg, subcutaneous (s.c.) (free-base form); this dose is considered to be within the rewarding range of the dose–response function that also elicits a behavioral response in adolescent C57BL/6J mice [28,32,33]. Peripheral injections were administered at a volume of 10 mL/kg.

Adolescent Injection Schedule

Beginning on PND 38, the first set of male and female mice were randomly subdivided into four experimental groups: (1) Control (saline s.c., vehicle i.p.), (2) NIC (0.36 mg/kg nicotine

s.c., vehicle i.p.), (3) WIN (saline s.c., 2 mg/kg WIN i.p.), and (4) NIC/WIN (0.36 mg/kg nicotine s.c., 2 mg/kg WIN i.p.). Saline and vehicle were the solutions used to dissolve nicotine and WIN, respectively. Mice received once daily injections for 12 consecutive days from PND 38 to PND 49. This timeframe is considered mid-adolescence in rodents, corresponding to ~13–17 in human years [26]. This represents a dynamic developmental period for both the endogenous nicotinic acetylcholine and cannabinoid systems; for instance, the highest level of CB1 receptor expression is found during this period [11,25,27,28,34]. The daily injection schedule was selected to model an experimental pattern of adolescent exposure as previously described [28]. Body weight was recorded prior to each injection. The second set of male and female mice were treated as above, but they were subdivided into the following experimental groups: (1) Control (saline s.c., vehicle i.p.), (2) LdWIN (saline s.c., low dose [0.2 mg/kg] WIN i.p.), and (3) NIC/LdWIN (0.36 mg/kg nicotine s.c., 0.2 mg/kg WIN i.p.). All above groups were tested in multiple smaller cohorts to enhance rigor and reproducibility of the findings. The current studies were designed to systematically assess changes following adolescent exposure under the varying conditions by maintaining precise dosing conditions via peripheral injections.

Intravenous Nicotine Self-Administration

Mice were mildly food restricted to 85%–90% of their free-feeding body weight and trained to press a lever in an operant chamber (Med Associates, St. Albans, VT) for food pellets (20 mg; TestDiet, Richmond, IN) under a fixed-ratio 5, time out 20 seconds (FR5TO20 sec) schedule of reinforcement. We have previously shown that these adolescent exposure

groups do not differ in operant food learning [28]. Once stable responding was achieved (>25 pellets per session across three subsequent sessions), subjects were surgically catheterized as previously described [21,35]. Briefly, mice were anesthetized with an isoflurane (1%–3%)/oxygen vapor mixture and prepared with intravenous catheters. Catheters consisted of a 6 cm length of silastic tubing fitted to guide cannula (Plastics One, Roanoke, VA) bent at a curved right angle and encased in dental acrylic. The catheter tubing was passed subcutaneously from the animal's back to the right jugular vein, and a 1 cm length of the catheter tip was inserted into the vein and tied with surgical silk suture. Following the surgical procedure, animals were allowed ≥48 hours to recover from surgery, then provided access to again respond for food reward. Mice were then permitted to acquire intravenous nicotine self-administration during 1 hour daily sessions, 6–7 days per week, at the standard training dose of nicotine (0.03 mg/kg/infusion). Nicotine was delivered through tubing into the intravenous catheter by a Razel syringe pump (Med Associates). Each session was performed using two retractable levers (one active, one inactive). Completion of the response criteria on the active lever resulted in the delivery of an intravenous nicotine infusion (0.03 mL infusion volume; FR5TO20 sec schedule). Responses on the inactive lever were recorded but had no scheduled consequences. Catheters were flushed daily with physiological sterile saline solution (0.9%, w/v) containing heparin (10 USP units). Catheter integrity was tested with the short-acting barbiturate anesthetic Brevital (methohexital sodium, Eli Lilly, Indianapolis, IN). Subjects and their data were removed from the study due to death or if the catheter integrity was compromised as determined by visual leakage or Brevital assessment. Behavioral responses were automatically recorded by MedAssociates software.

Experimental Design

The experimental design is outlined in Figure 2.1. Following adolescent injections, mice remained drug-free until adulthood (PND 70). Thereafter, they were examined for differences in cognitive behavior as reported previously [28]. For these investigations, to ascertain the dose–response function, mice were tested according to the established mouse intravenous self-administration protocol [21]. Following an acquisition period of at least 7 days on the training dose (0.03 mg/kg/infusion), the animals were presented with a different dose of nicotine for at least 5 days, and the mean intake for the last two sessions was used for statistical analyses. In between each dose, subjects were returned to the 0.1 mg/kg/infusion dose for 2 days or until intake returned to baseline levels. The dose–response function occurred over a total ~35 sessions with testing sessions occurring 6 days per week. Thereafter, mice were stabilized on the moderate 0.1 mg/kg/infusion dose across three baseline sessions after successfully passing the Brevital catheter patency test. Then, subjects were challenged with an injection of the low dose WIN (0.2 mg/kg) or vehicle control, 20 minutes prior to the nicotine self-administration session. Injections of vehicle or low dose WIN were administered in a random, counterbalanced design both within and across groups, and subjects were permitted at least 2 baseline days in between WIN/vehicle administration to return to baseline levels of nicotine intake. After the crossover experiment with the single, acute dose of WIN, mice were chronically pretreated with the same low WIN dose prior to each session across seven consecutive sessions, and nicotine intake on the seventh session was used to determine the effects of chronic coexposure during adulthood

for all groups. Since the control groups (adolescent vehicle treatment) for the moderate and low dose WIN cohorts exhibited similar effects with pretreatment, data were compiled into one graph for each sex. Finally, mice were again returned to self-administer the 0.1 mg/kg/infusion dose, and after achieving baseline levels of responding, they were then transitioned to respond for saline infusions (no nicotine). Eleven mice were required to be excluded due to death/cannibalization by cagemates (three female Control, one female NIC, three female and one male NIC/WIN, two female NIC/LdWIN, and one male LdWIN), and six were excluded due to compromised catheter integrity (two female Control, two female NIC, one female NIC/LdWIN, and one male LdWIN).

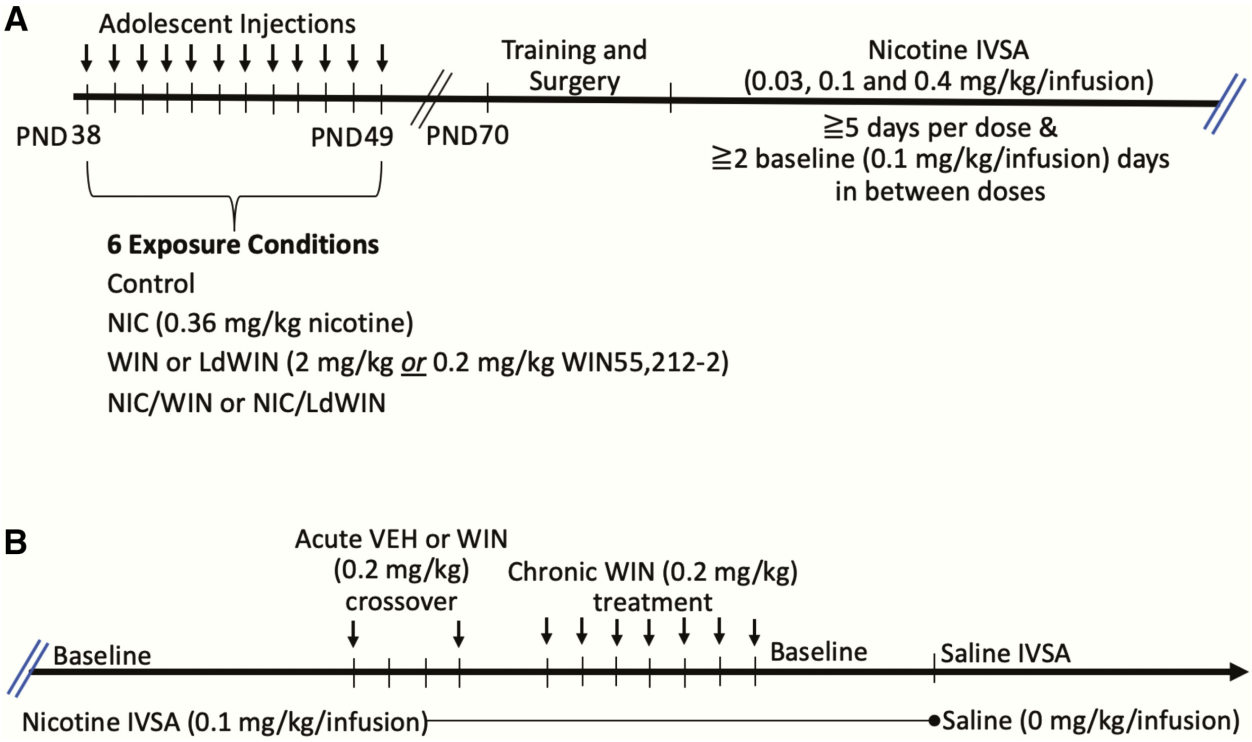

Figure 2.1 Schematic outline of the experimental design.
(A) All mice were treated in adolescence with nicotine (NIC), WIN55,212-2 (WIN), NIC/WIN coexposure, or vehicle (VEH) control. WIN was administered at either a low or high dose for the single and coexposure conditions. After PND 70, mice began testing for subsequent examination of intravenous nicotine self-administration (IVSA) across doses. (B) After

reestablishing baseline responding on the 0.1 mg/kg/infusion nicotine dose, mice were then pretreated across sessions with vehicle or low dose WIN in a crossover design. Thereafter, mice were examined with chronic low dose WIN and nicotine self-administration coexposure for seven consecutive testing sessions. Finally, after again reestablishing baseline responding, mice were transitioned to respond for saline infusions in the absence of nicotine. PND = postnatal day.

Statistical Analyses

Given that these studies sought to investigate the effects of drug exposure relative to the control condition within each sex, statistical comparisons were performed separately for males and females based on this a priori hypothesis [28]. Data were analyzed by a t test, one-way or two-way analysis of variance (ANOVA) with Prism 7 software (GraphPad, La Jolla, CA), as appropriate. Data obtained across sessions were analyzed with a repeated measures two-way ANOVA. Significant main or interaction effects were followed by Bonferroni post hoc comparison with correction for multiple comparisons. The criterion for significance was set at $\alpha = 0.05$.

RESULTS

Intravenous Nicotine Self-Administration During Adulthood

Adolescent exposure groups were examined for differences in nicotine intake during adulthood across low, moderate, and high self-administration doses (Figure 2.1A). This approach allows for the assessment of the dose–response function, which provides a measure of responding across nicotine doses with increasing value of reinforcement (ascending limb of the dose–response) and doses inducing greater aversion and/or satiation (descending limb of the dose–response) [21]. In male mice, significant differences were

found on the ascending limb at the 0.03 mg/kg/infusion dose, but not at higher doses (Figure 2.2A) (repeated measures two-way ANOVA, Group $F(3,25) = 2.13$, $p = .122$; Dose $F(3,75) = 38.15$, $p < .0001$; Interaction $F(9,75) = 2.29$, $p = .024$). Specifically, the WIN and nicotine/WIN adolescent exposure groups exhibited a significantly increased number of nicotine infusions compared with the control and nicotine adolescent exposure groups ($p < .05$ for WIN compared with either control or nicotine; $p < .01$ for nicotine/WIN compared with either control or nicotine). Further, the groups did not differ in their saline level of responding, indicating that these differences were not due to a general increase in lever pressing behavior. Since both the WIN and nicotine/WIN exposure conditions involved a moderate dose of the cannabinoid agonist (2 mg/kg), we next addressed the possibility that this WIN dose could have masked the effects of nicotine in an interactive effect. Thus, we examined a separate cohort of mice exposed to a low dose of WIN (0.2 mg/kg), either in the presence or absence of nicotine. However, differences were not found in the dose–response function among these adolescent treatment conditions, with all groups exhibiting a main effect for nicotine dose (Figure 2.2B) (repeated measures two-way ANOVA, Group $F(2,19) = 1.06$, $p = .368$; Dose $F(3,57) = 15.51$, $p < .0001$; Interaction $F(6,57) = 0.845$, $p = .541$).

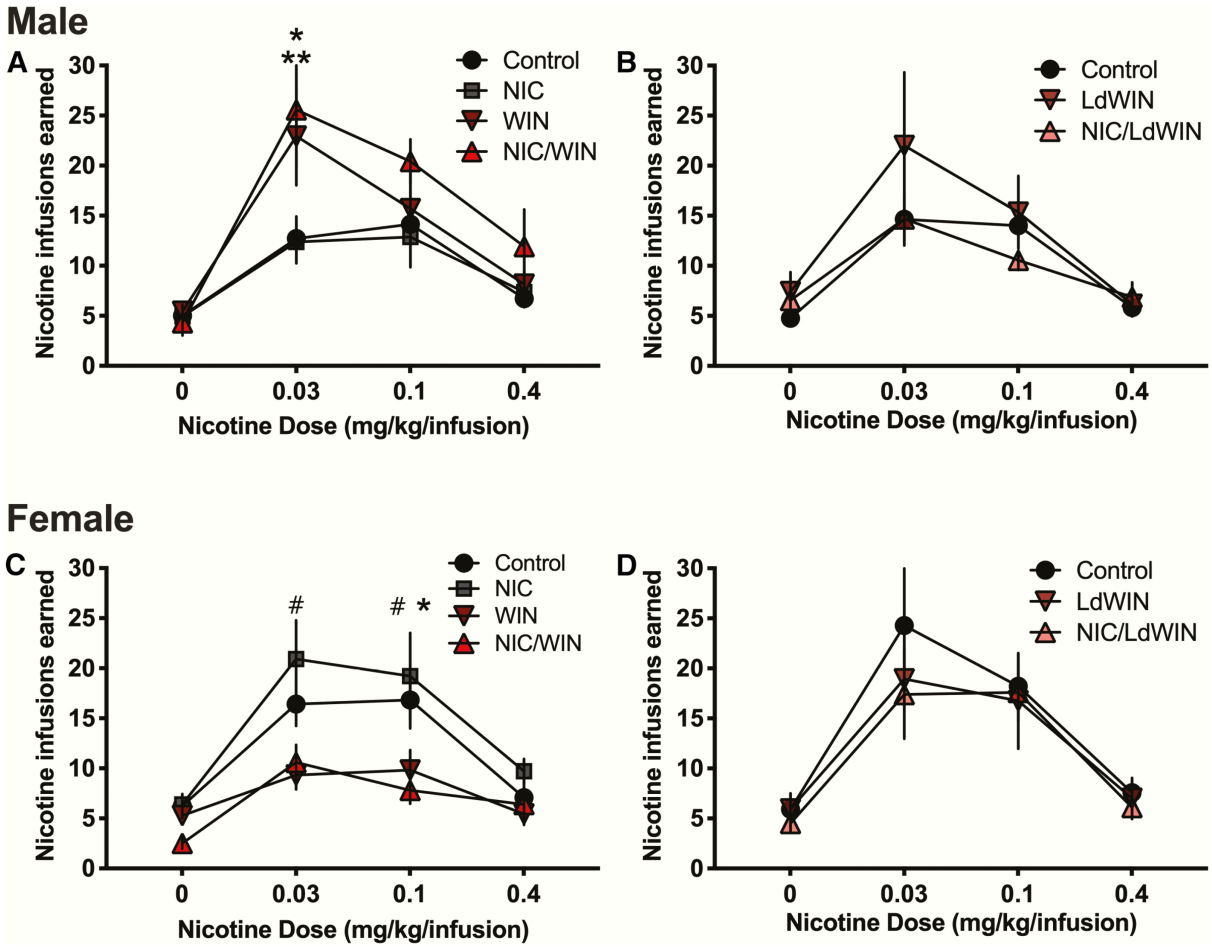

Fig 2.2 Male and female mice exposed to the cannabinoid agonist during adolescence exhibit opposing effects on nicotine self-administration in adulthood. (A and B) Male intravenous nicotine self-administration dose–response function. (A) Following exposure to the cannabinoid agonist WIN (2 mg/kg) during adolescence, adult male mice demonstrated an increase in nicotine intake on the ascending limb of the dose–response function at the 0.03 mg/kg/infusion dose compared with the vehicle control and nicotine only (NIC) groups. A similar increase in nicotine intake was also found with nicotine and WIN coexposure (NIC/WIN) at this dose compared with both the vehicle and NIC groups (n = 6–8/group). *p < .05 Control vs. WIN, and NIC vs. WIN; **p < .01 Control vs. NIC/WIN, and NIC vs. NIC/WIN. (B) Adult male mice administered the lower dose of WIN (0.2 mg/kg), either in the presence or absence of nicotine, during adolescence did not exhibit statistically significant differences from the control across the dose–response function (n = 7–8/group). (C and D) Female intravenous nicotine self-administration dose–response function. (C) Adult female mice exposed to nicotine (NIC) during adolescence earned significantly more nicotine infusions at the low (0.03 mg/kg/infusion) and moderate (0.1 mg/kg/infusion) doses, as compared with the adolescent-exposed cannabinoid agonist WIN (2 mg/kg) groups, either alone or with nicotine coexposure. The coexposure WIN and nicotine group also earned significantly less nicotine infusions than the control condition at the moderate 0.1 mg/kg/infusion dose (n = 6–9/group). #p < .01 NIC vs. WIN or NIC/WIN; *p < .05 Control vs. NIC/WIN. (D) Adult female

mice administered the low dose of WIN during adolescence, alone or with nicotine, did not differ from the control vehicle group across the dose–response function (n = 6–8/group).

In female mice, statistically significant main and interaction effects were found among the control, nicotine, WIN, and coexposure nicotine and WIN groups (Figure 2.2C) (repeated measures two-way ANOVA, Group $F(3,24) = 5.24$, $p = .006$; Dose $F(3,72) = 33.44$, $p < .0001$; Interaction $F(9,72) = 2.82$, $p = .007$). The post hoc analysis revealed an upward shift in the dose–response function for the nicotine exposure group, as compared with both the WIN and coexposure nicotine and WIN groups. Specifically, at the 0.03 mg/kg/infusion dose, the adolescent nicotine group exhibited a significantly greater number of nicotine infusions than the adolescent WIN ($p < .001$) and nicotine/WIN coexposure ($p < .01$) groups. At the moderate 0.1 mg/kg/infusion dose, the nicotine group also demonstrated a statistically significant increase from the WIN group ($p < .01$) and nicotine/WIN coexposure group ($p < .001$), and the nicotine/WIN coexposure group was also significantly decreased compared with the control group ($p < .05$). No other groups significantly differed from the control, or at the saline and high dose of nicotine (0.4 mg/kg/infusion). Thereafter, a second set of female mice were examined for differences with the lower dose of WIN. However, the low dose WIN adolescent exposure groups, either in the presence or absence of nicotine, did not differ across the dose–response function from the control condition, with a significant main effect of dose evidenced (Figure 2.2D) (repeated measures two-way ANOVA, Group $F(2,18) = 0.42$, $p = .662$; Dose $F(3,54) = 29.26$, $p < .0001$; Interaction $F(6,54) = 0.45$, $p = .842$).

Interactive Effects of Acute or Chronic WIN Exposure During Adult Nicotine Self-Administration

To examine whether further exposure in adulthood to a cannabinoid agonist subsequently alters nicotine intake, mice were pretreated with the low dose of the cannabinoid agonist or vehicle prior to a nicotine self-administration session; thereafter, the mice were then repeatedly administered the low dose of the cannabinoid agonist prior to seven consecutive nicotine self-administration sessions (Figure 2.1B). In adult males, we found that both acute and chronic treatment with WIN significantly attenuated nicotine intake relative to the vehicle control (repeated measures one-way ANOVA, $F(2,32) = 8.09$, $p = .001$; Post hoc, vehicle vs. acute $p < .05$, vehicle vs. chronic $p < .01$) (Figure 2.3A), indicating that cannabinoid co-use in adulthood reduces nicotine consumption. Interestingly, when we examined the adolescent-exposed nicotine and WIN groups, a stark contrast in responding was evidenced. Across all adolescent drug groups, the cannabinoid agonist was ineffective in altering nicotine intake relative to infusions earned following vehicle injection (repeated measures one-way ANOVAs: Nicotine, $F(2,14) = 0.37$, $p = .695$; WIN, $F(2,16) = 0.61$, $p = .554$; Low dose WIN, $F(2,12) = 5.77$, $p = .018$; Nicotine and WIN coexposure, $F(2,8) = 1.75$, $p = .234$; Nicotine and low dose WIN coexposure, $F(2,14) = 2.67$, $p = .104$) (Figure 2.3B, C, D, E, and F, respectively).

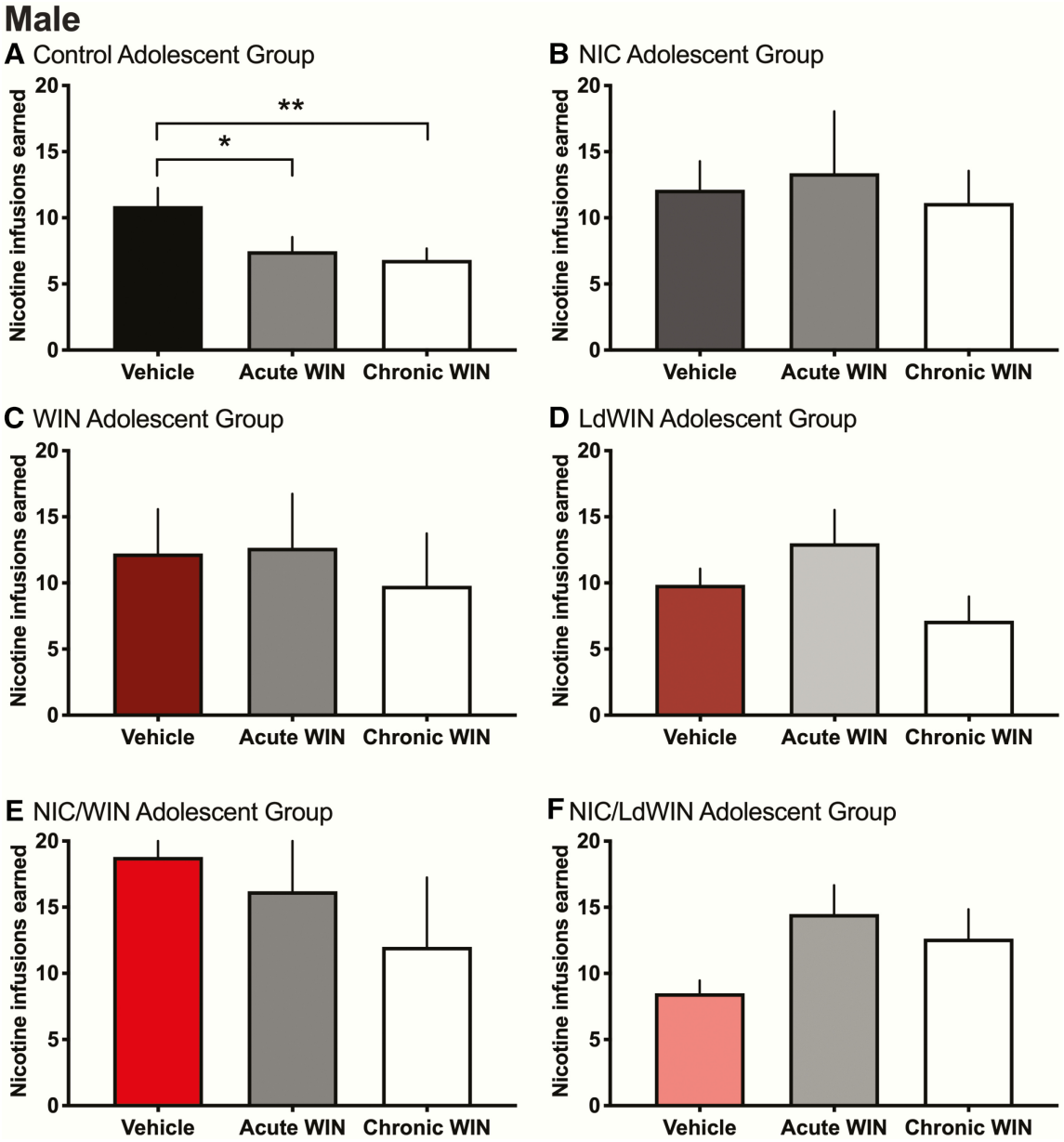

Fig 2.3 Adolescent drug exposure in male mice results in resistance to the effects of the cannabinoid agonist on nicotine intake in adulthood.
(A) Control male mice exhibit a statistically significant reduction in nicotine intake after acute cannabinoid agonist preadministration and following 7 consecutive days of treatment (chronic) in adulthood (n = 17). *p < .05, **p < .01 compared with vehicle injection. (B–F) Administration of a low dose of the cannabinoid agonist during adulthood was ineffective in altering nicotine intake in male mice exposed to the following during adolescence: (B) nicotine (n = 8), (C) the cannabinoid agonist WIN (n = 9), (D) low dose of the cannabinoid agonist WIN (n = 7), (E) coexposure of nicotine and the cannabinoid agonist WIN (n = 5), or (F) coexposure of nicotine and the low dose of the cannabinoid agonist WIN (n = 8). Data represent mean values ± standard error of the mean (SEM).

In adult females, the control group exhibited a similar effect of cannabinoid agonist pretreatment in reducing nicotine intake as to that found in the males (repeated measures one-way ANOVA, $F(2,26) = 15.94$, $p < .0001$) (Figure 2.4A). Specifically, in post hoc analyses, the vehicle condition exhibited a higher level of nicotine infusions compared with pretreatment with the cannabinoid after one session (acute, $p < .05$) and after seven consecutive sessions (chronic, $p < .0001$). Further, chronic administration of the cannabinoid agonist significantly reduced nicotine intake to a greater extent than the acute condition ($p < .05$). However, adolescent drug exposure resulted in a resilience to the effects of the cannabinoid agonist during adulthood on nicotine intake, since differences were not found in the number of nicotine infusions earned after cannabinoid agonist injection for all other groups (repeated measures one-way ANOVAs: Nicotine, $F(2,12) = 3.09$, $p = .083$; WIN, $F(2,16) = 2.16$, $p = .148$; Low dose WIN, $F(2,14) = 2.11$, $p = .158$; Nicotine and WIN coexposure, $F(2,10) = 2.11$, $p = .173$; Nicotine and low dose WIN coexposure, $F(2,10) = 0.27$, $p = .771$) (Figure 2.4B, C, D, E, and F, respectively).

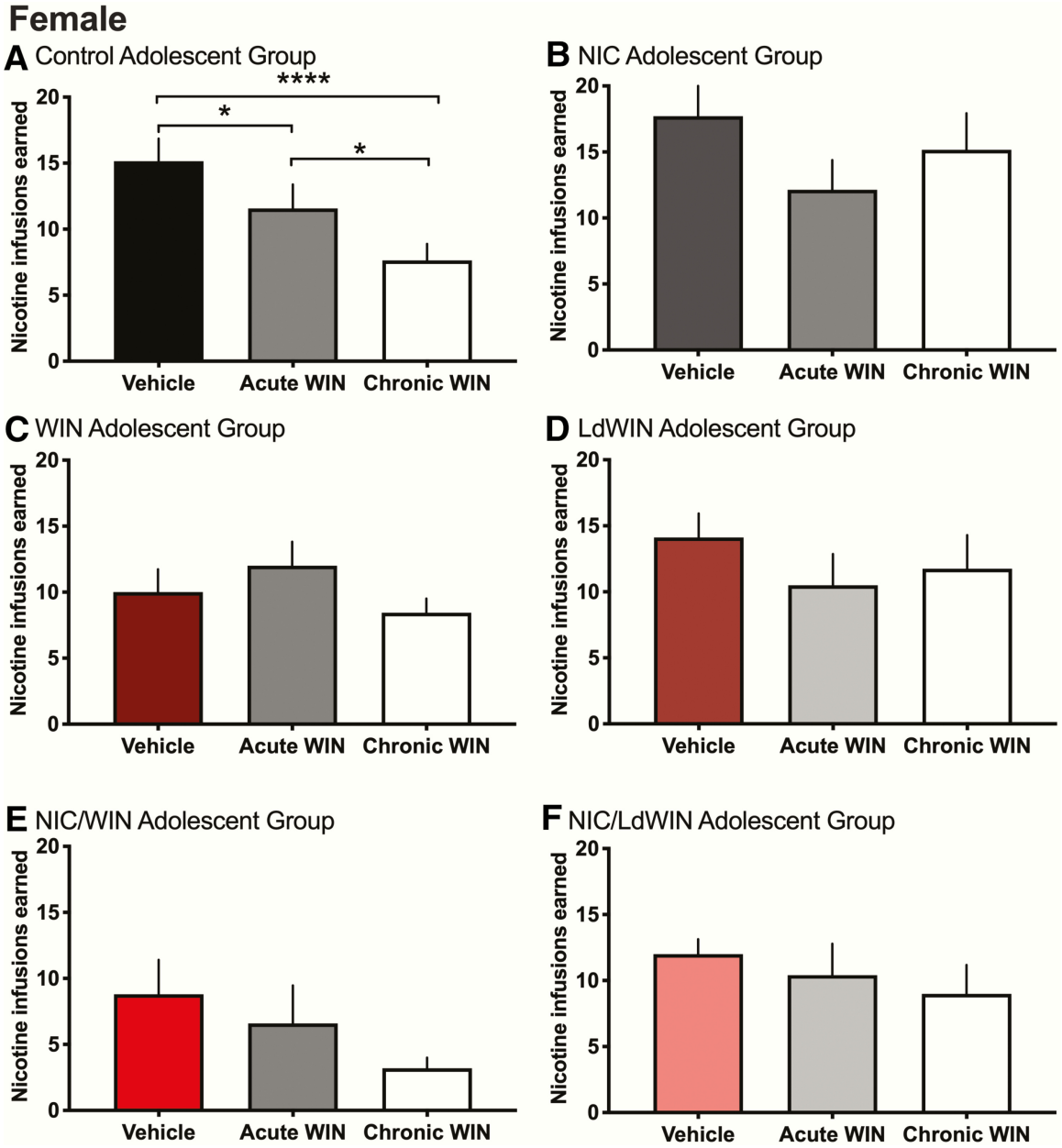

Fig 2.4 Adolescent nicotine or cannabinoid agonist exposure in female mice prevents the lowering effect of the cannabinoid agonist on nicotine intake in adulthood.
(A) Control female mice earned significantly fewer nicotine infusions both after an acute cannabinoid agonist preadministration and with chronic exposure (n = 14). *p < .05 for vehicle versus acute WIN, and acute WIN versus chronic WIN, ****p < .0001 for vehicle versus chronic WIN. (B–F) The cannabinoid agonist was ineffective in altering nicotine intake during adulthood in female mice with adolescent exposure to (B) nicotine (n = 7), (C) the cannabinoid agonist WIN (n = 9), (D) low dose of the cannabinoid agonist WIN (n = 8), (E) coexposure of nicotine and the cannabinoid agonist WIN (n = 6), or (F) coexposure of nicotine and the low dose of the cannabinoid agonist WIN (n = 6). Data represent mean values ± standard error of the mean (SEM).

DISCUSSION

In these studies, we found that adolescent cannabinoid and/or nicotine exposure exert a lasting impact on susceptibility to drug reinforcement, which is evidenced in adulthood. However, these effects were dependent on the substance of abuse (cannabinoid agonist or nicotine), dose of the cannabinoid, and sex. Specifically, adult males exhibited increased nicotine self-administration at the lower rewarding nicotine dose following adolescent cannabinoid agonist exposure at the moderate dose (2 mg/kg), either alone or with nicotine coadministration. In contrast, adult females demonstrated an opposing effect following adolescent cannabinoid exposure at the moderate dose, in which such exposure resulted in decreased nicotine intake compared with nicotine exposure alone. However, differences were not induced within either sex with adolescent exposure to the lower dose of the cannabinoid agonist (0.2 mg/kg). Furthermore, after maintaining nicotine self-administration, pretreatment with the low dose of the cannabinoid agonist attenuated nicotine intake in both male and female control mice, and this lowering effect was evidenced both acutely and after chronic pairings. Surprisingly, the cannabinoid agonist was ineffective in altering nicotine intake in mice previously exposed to nicotine, the cannabinoid agonist, or both during adolescence; an effect that was present at both the lower and moderate doses of the cannabinoid agonist.

Impact of Adolescent Drug Exposure on Adult Nicotine Intake

Nicotine self-administration produces an inverted U-shaped dose–response curve, which represents the competing positive and negative properties of nicotine. The increased

responding for nicotine over the ascending limb of the curve reflects the increasing reinforcing effects of nicotine as the unit dose increases. In contrast, the decreased responding over the descending limb of the curve reflects the increasing aversive properties of nicotine or satiation. Mesolimbic dopamine neurons have been primarily implicated in modulating the rewarding and reinforcing aspects of the drug, [36] whereas the aversive signaling of nicotine appears to involve the habenulo-interpeduncular pathway [12]. Our findings suggest that adolescent cannabinoid exposure most likely alters the function of the mesolimbic pathway, as differences were found primarily on the ascending limb of the dose–response function. In support of this notion, adolescent cannabinoid or nicotine exposure has previously been shown to alter monoaminergic signaling [37–40]. However, in our study, nicotine alone was ineffective in altering later drug-taking behaviors in males, either in combination with the cannabinoid or alone. Since studies have shown that of those adolescents age 12–17 who smoke, the majority smoke one or less than one cigarette per day (50.1%), [41] the current studies focused on a rewarding dose with once daily exposure of a rewarding dose [28,32,33]. Thus, the current results have particular relevance to experimental patterns of drug consumption found in youth.

Differential patterns of expression of the cannabinoid receptors are found across adolescent development and between males and females, [42] and CB1Rs exhibit highest level of expression during the developmental period of mid-adolescence (PND 25–50) [42]. Thus, the potential for exogenous cannabinoids to alter synaptic and neural circuit function may be considered greatest during this time period. Indeed, prior studies have revealed an effect of CB1R activation on adolescent brain development and indicate a correlation between adolescent exposure and later cognition and reward-related function. For instance,

we found that adult males exposed during adolescence to the moderate 2 mg/kg dose of WIN exhibited increased cognitive flexibility in a learning reversal task, decreased anxiety-associated behaviors, and increased natural reward consumption with the same exposure paradigm [28]. The coexposure condition of both nicotine and the moderate dose of WIN also led to similar behavioral profiles as WIN alone in these measures, [28] suggesting that a potentiative or additive effect was not present similar to that found in the current studies with nicotine intake. With regard to females, they were found to be overall more resistant to the long-term effects of adolescent drug exposure, in which the moderate dose WIN females exhibited decreased natural reward consumption compared with the control females [28]. Interestingly, CB1R knockout mice are resistant to nicotine-mediated locomotion and conditioned place preference, but do not differ in nicotine self-administration, as compared with wild-type mice, [43,44] which suggests that the lack of CB1Rs during adulthood may affect generalized locomotor behavior and drug-conditioned memory function, but perhaps not the motivation to consume nicotine. However, given the constitutive knockout of the gene in these mice, it is possible that compensatory mechanisms occurred during development, resulting in altered expression of other receptors, potentially including cannabinoid 2 receptors and/or nAChRs.

Adolescent Exposure Infers Resistance to a Cannabinoid-Induced Decrease in Nicotine Intake

Both single and co-use of nicotine and cannabinoid products are prevalent during adolescence and adulthood. Thus, we further examined coexposure during adulthood, under

both control conditions and following adolescent drug exposure. In the control group, we found that the low dose of the cannabinoid agonist reduced nicotine intake in adulthood. This represents the first demonstration of the effects of a cannabinoid agonist on intravenous nicotine self-administration in mice. These results were surprising since the CB1 receptor antagonists rimonabant and taranabant have also been shown to reduce nicotine consumption [45]. However, when one considers that additive effects may be induced on brain reward circuitries, such as that found with reduced brain stimulation thresholds in the presence of rewarding doses of nicotine, it is likely that the presence of the cannabinoid agonist augmented the activity of the reward circuits in the brain, leading to a reduction in nicotine intake while maintaining similar circuit activation to support drug reinforcement. However, this stipulation will need to be more directly tested in future studies.

We further found that the effectiveness of the cannabinoid agonist in reducing nicotine self-administration is dependent on prior drug exposure during adolescence, as all of the adolescent nicotine and/or WIN exposure groups did not differ in nicotine intake with WIN pretreatment in adulthood. Of further note, we found that this lack of responsiveness to the dampening effects of the cannabinoid agonist on nicotine intake also occurred in adolescent groups exposed to the low dose of the cannabinoid agonist. It is important to note that this level of exposure did not induce any other detectable behavioral effects during adulthood, either in this study or in our prior analysis of cognitive, anxiety-, and depression-associated behaviors [28]. Given these findings, it is possible that patients may differentially respond to pharmacotherapeutics based on developmental drug exposure, representing a potential underlying factor mitigating individual differences in cessation outcomes. Indeed,

given that we found differences in nicotine intake during adulthood with developmental drug exposure, and currently available pharmacotherapeutics such as varenicline also target nAChRs, similar signaling mechanisms may be involved in mitigating the behavioral responses to these drug compounds.

Finally, in these studies, we examined the effects of an injected cannabinoid agonist during adolescent development on nicotine self-administration in adulthood. Importantly, these results have direct implications for the use of "spice" synthetic cannabinoids, of which the majority belong to the aminoalkylindole class, including WIN55,212-2 [7–9]. In addition, these findings likely have further implications for cannabis exposure. Δ9-Tetrahydrocannabinol (THC) has been characterized as a partial agonist of the CB1R, and therefore, it is possible that the low dose of the WIN agonist could have resulted in the occupation of a fewer number of receptors, thereby inducing an effect more similar to a higher dose of a partial agonist on downstream cellular signaling. However, this will need to be more systematically addressed in future studies. Moreover, while it is possible that volitional intake during adolescence may differentially alter drug reinforcement, rather than experimenter administered injections, there are some caveats to such an experimental design. First, it is not yet feasible to implant intravenous catheters in adolescent mice. Second, the dose that each animal receives cannot be discretely controlled with self-administration studies. This point is further compounded by the fact that coexposure conditions result in different intake amounts of each drug, as compared with single use conditions. Furthermore, both THC and WIN self-administration in rodent models have been difficult to establish in many labs, although some have been successful due to specific doses and reinforcement testing paradigms [31,46,47]. In particular, for THC in rats, the combined

presence of cannabidiol sustained self-administration behavior in both intravenous and vapor paradigms [47,48]. This is interesting given that many THC e-cigarette vapes on the market do not contain cannabidiol, at least as indicated on commercial packaging. Given these considerations and with the foundational findings derived from the current studies, it will nevertheless be important in future studies to develop models for volitional adolescent nicotine and cannabinoid self-administration, perhaps via vapor exposure, and then to determine whether the variable, self-titrated levels of each drug differentially impacts nicotine and/or cannabinoid self-administration in adulthood.

CONCLUSIONS

In these studies, we have found that adolescent cannabinoid and/or nicotine exposure leads to differential effects on nicotine-taking behaviors in male and female mice. Further, such developmental exposure appears to alter the brain's later responsiveness with important implications for co-use conditions, in which developmental cannabinoid or nicotine exposure leads to sustained use of nicotine with cannabinoid coexposure in adulthood. In future studies, it will be important to examine both self-administered nicotine and cannabinoid exposure during adolescence and throughout the transition from adolescence to adulthood, as well as adolescent nicotine and cannabinoid exposure on other aspects of nicotine dependence, including withdrawal and relapse-related reinstatement behaviors. It will also be important to assess whether the impact of adolescent exposure differs due to genetic factors mitigating vulnerability. For instance, it has been demonstrated that humans with allelic variation in the catechol-O-methyltransferase gene are more likely to develop

schizophrenia-related symptomology following adolescent cannabinoid use, [49] a finding that is of further relevance given the very high comorbidity found between schizophrenia and nicotine dependence [50]. In sum, given the increased adolescent use of nicotine and THC containing vape pens, along with the availability of cannabis and synthetic "spice" products, the long-term consequences of developmental drug exposure represent an important health issue, and as such, the current findings should serve to guide future policy efforts to limit youth exposure.

Supplementary Material

A Contributorship Form detailing each author's specific involvement with this content, as well as any supplementary data, are available online at https://academic.oup.com/ntr.

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Chapter 3: Edibles, Vaping, and Relapse: Examining the Lasting Impact of Adolescent Exposure to THC and/or Nicotine on Incubation of Nicotine Craving

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ABSTRACT

The increased prevalence of nicotine and cannabis use among adolescents represents a significant health and societal concern. While long-term behavioral and cognitive effects have been documented, the extent of this impact across the various facets of drug dependence and with varying routes of exposure is largely unknown. Here, we sought to examine whether edible THC and/or e-cigarette nicotine vape during adolescence leads to persistent changes in adult drug seeking behavior. Male and female adolescent mice were administered either nicotine (subcutaneous or aerosolized vape), THC (oral), or co-exposed to both nicotine vape and oral THC, and then examined for intravenous nicotine self-administration and subsequent incubation of craving. We found that males and females with an adolescent history of THC consumption self-administered higher levels of nicotine in adulthood than the respective control group. A pronounced incubation of craving response was evident in the control mice. However, males and females exposed to nicotine in adolescence exhibited a lack of an incubation effect dependent on the route of adolescent administration. Further, females with an adolescent history of the higher dose of THC may have an overall increased level of responding which also impacted their incubation of nicotine craving. Finally, adolescent nicotine vape and THC co-exposure appeared to have counteracted the effects of these drugs alone, as subjects with a history of nicotine vape and THC exposure displayed a robust incubation of craving response. These findings reveal that adolescent drug use impacts later reward-associated behaviors, with important implications for the motivational effects of conditioned cues on drug relapse.

INTRODUCTION

Nicotine dependence remains the leading cause of preventable death worldwide with over 1.3 billion current users [1]. Nicotine can be consumed in many forms including tobacco cigarettes and in recent years, electronic nicotine delivery systems (ENDS). ENDS involve the heating of nicotine-containing liquid to produce an aerosol that users inhale similarly to tobacco smoke. The use of ENDS, also known as e-cigarettes or nicotine vape pens, has drastically increased over the past decade, especially among youth, whereas tobacco cigarette use has declined [2, 3]. Of further concern, adolescent nicotine exposure increases the risk of developing nicotine dependence and other substance use disorders, including cannabis use disorder [4, 5].

Cannabis is the most abused illicit drug with over 200 million people using it around the world [6]. The main psychoactive component in cannabis is Δ9-tetrahydrocannabinol (THC). THC is consumed by humans in many forms including orally in THC-containing foods or drinks known as edibles. Edible THC is appealing to many users because it can be consumed without the smoke of traditional cannabis use and the lipids in food make drug absorption easier [7]. When consumed orally, it has been shown to take at least 30 minutes to see a significant rise in blood THC levels and the gradual return to baseline lasts six hours post-ingestion in humans [8]. Because edibles require a longer time before users begin to feel the effects and the concentrations of THC in these products can be much higher, it is easy for inexperienced users, particularly youth, to consume more THC than intended [9].

Beyond single drug use, co-use of multiple drugs like both cannabis and nicotine-containing products is frequent. Around 60% of cigarette smokers report ever using

cannabis, and 90% of cannabis users report ever smoking cigarettes in their lifetime [10]. Youth and young adults who co-use both report consuming more cannabis and nicotine annually [11]. People suffering from these individual and co-occurring substance use disorders may try to abstain from taking the drugs, but relapse is a major concern as most people begin smoking again within the first week [12].

Drug relapse and craving during abstinence has been shown to be triggered by certain cues associated with drug-taking [13-15]. In humans, nicotine craving in response to nicotine-associated cues increases over time during abstinence [16]. These cues can include the physical environment, people with whom the drug taking typically occurs, as well as any associated visual, auditory, and olfactory sensory perceptions [17]. This behavior is depicted in animal models of research as the incubation of craving paradigm in which cue-induced drug-seeking behavior increases over time during abstinence following drug self-administration. In rats, this has been demonstrated for several drugs of abuse, including heroin, alcohol, and nicotine [18, 19]. Although these studies have established important findings for single drug use, it remains to be determined whether polydrug use alters the incubation response and whether similar effects are found for the incubation of nicotine craving in mice.

The studies outlined below examined how this incubation of nicotine craving behavior, as well as operant learning and later drug intake, are altered dependent on prior drug exposure. Specifically, adolescent male and female mice were exposed to nicotine via injections or vapor, a lower or higher dose of THC, or both, and assessed in adulthood for differences in operant learning, nicotine intake, and relapse-related drug seeking behaviors.

The two routes of administration for nicotine are used because injections have been one of the most common methods of drug exposure in adolescent mice, which allows for comparisons to other studies, whereas the nicotine vapor exposure is highly translational to the current use of ENDS products. Moreover, two doses of THC are administered to account for dose-dependent effects of oral consumption. We proposed that adolescent single and poly-drug exposure alters later nicotine intake and susceptibility to cue-induced relapse in adulthood. The findings from this study can inform how previous drug history can impact the effectiveness of relapse interventions for those in the early stages of abstinence.

METHODS

Animals

Male and female wildtype C57BL/6J mice were derived from breeders in our laboratory animal facilities. In total 194 mice were assessed in these studies. Mice were maintained in an environmentally controlled vivarium on a 12 h reversed light/dark cycle. Food and water were provided ad libitum until postnatal day 70, at which time subjects were mildly food restricted to 85–90% of their free-feeding bodyweight for behavioral assessments, while water was continued to be provided ad libitum. All experiments were conducted in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at the University of California, Irvine.

Drugs

For the nicotine injections, (-)-Nicotine hydrogen tartrate salt (MP Biomedicals) was dissolved in 0.9% sterile saline and adjusted to pH 7.4. Nicotine was administered at a dose of 0.36 mg/kg, subcutaneous (s.c.) (free-base form); this dose is considered to be within the rewarding range of the dose response function that also elicits a behavioral response in adolescent C57BL/6J mice [20]. Peripheral injections were administered at a volume of 10 mL/kg. For the aerosolized vape nicotine, (-)-Nicotine hydrogen tartrate salt was dissolved in 50% propylene glycol and 50% glycerin (PG:VG) for the 7.5mg/mL concentration and adjusted to pH 7.4 . Our prior study has shown that this concentration elicits significant levels of the nicotine metabolite, cotinine, in the blood of rodents exposed to nicotine vapor [21]. Nicotine or vehicle vapor was administered using LJARI vapor chambers (23 cm W x 20.5 cm H x 35.5cm L) which were programmed to administer 12 puffs of vapor across a one-hour session. Each vapor administration was programmed for five seconds and air flow was regulated at 1 L/min. THC in ethanol was obtained from the NIH NIDA Drug Distribution Program. Ethanol was first evaporated out under nitrogen and diluted in sesame oil vehicle for oral gavage administration at a volume of ~0.1 ml, adjusted based on body weight. The oral 5 and 10 mg/kg doses are considered moderate and moderately high doses, with other reports in the literature examining up to 20 mg/kg without significant adverse effects [PMID 34652500]. The oral 5 and 10 mg/kg doses have been found to be behaviorally effective in reducing locomotion to 20-50% of baseline levels, and mice will readily consume THC orally at these doses [22]. For these studies, the THC was diluted at 5 mg/kg or 10 mg/kg in sesame oil and was administered via oral gavage at a concentration of 1 mg/ml and 2 mg/ml respectively.

Adolescent Drug Exposure Schedule

The experimental paradigm is outlined in **Figure 3.1A**. Beginning on postnatal day (PND) 38, male and female mice were randomly subdivided into seven experimental groups: (1) Control: saline injections (saline, subcutaneous) or oral sesame oil and vehicle vape, (2) NIC SC: nicotine injections (0.36 mg/kg nicotine, subcutaneous), (3) NIC Vapor: oral sesame oil and aerosolized nicotine (7.5 mg/ml nicotine), (4) THC: oral THC (5 mg/kg) and vehicle vape, (5) hTHC: oral THC (10 mg/kg) and vehicle vape, (6) THC/NIC: oral THC (5 mg/kg) and nicotine vapor (7.5 mg/mL nicotine), and (7) hTHC/NIC: oral THC (10 mg/kg) and nicotine vapor (7.5 mg/mL nicotine). Mice received treatments across 12 consecutive days from PND 38 to PND 49, and oral vehicle or THC was administered 30 min prior to the 1hr vape sessions. Body weight was recorded prior to each drug exposure. On PND 49, for cotinine analysis, blood was collected randomly from a subset of subjects from each nicotine-exposed experimental group (n = 37 males, 39 females) twenty minutes after the last nicotine injection or 1 hr vapor session. Subjects were all tested in multiple smaller cohorts with randomly assigned drug exposure conditions to enhance rigor and reproducibility of the findings.

Operant Food Training

On PND 70, subjects were mildly food restricted and trained to press a lever in an operant chamber (Med Associates) for food pellets (20 mg; TestDiet) under a fixed-ratio 5, time out 20 second (FR5TO20s) schedule of reinforcement. Each session was performed using two retractable levers (one active, one inactive) and a cue light to indicate reward delivery.

Completion of the response criteria on the active lever resulted in the delivery of a food pellet. Responses on the inactive lever were recorded but had no scheduled consequences. All behavioral responses were automatically recorded by MedAssociates software. Subjects were permitted to respond for food in daily sessions across eight days, and all subjects met the full food training criteria of earning more than 25 pellets across three consecutive sessions.

Intravenous Nicotine Self-Administration

Once stable responding was achieved during operant food training, subjects were put back on full food for a minimum of three days before being surgically catheterized as previously described [23]. Briefly, mice were anesthetized with an isoflurane (1%–3%)/oxygen vapor mixture and prepared with intravenous catheters. Catheters consisted of a 6 cm length of silastic tubing fitted to guide cannula (Plastics One) bent at a curved right angle and encased in dental acrylic. The catheter tubing was passed subcutaneously from the animal's back to the right jugular vein, and a 1 cm length of the catheter tip was inserted into the vein and tied with surgical silk suture. Following the surgical procedure, subjects were allowed ≥48 hours to recover from surgery, then provided access to again respond for food reward. Mice were then permitted to acquire intravenous nicotine self-administration (IVSA) during one-hour daily sessions, six days per week, at the standard training dose of nicotine (0.03 mg/kg/infusion) in the same operant chambers as used in food training. Nicotine was delivered through tubing into the intravenous catheter by a Razel syringe pump (Med Associates). Completion of the response criteria on the active lever resulted in the delivery

of an intravenous nicotine infusion (0.03 mL infusion volume; FR5TO20 sec schedule) and a cue light indicating reward delivery. Responses on the inactive lever were recorded but had no scheduled consequences. Mice are permitted to self-administer nicotine for eight days on the lower 0.03 mg/kg/infusion nicotine dose and five days on the moderate 0.1 mg/kg/infusion nicotine dose, which has previously been shown to permit consistent titration of nicotine responding in mice [23]. Behavioral responses were automatically recorded by MedAssociates software. Catheters were flushed daily with physiological sterile saline solution (0.9%, w/v) containing heparin (10 USP units).

Incubation of Craving

To assess nicotine seeking behavior during a state of abstinence, mice began the incubation of nicotine craving paradigm following the final day of intravenous nicotine self-administration at the 0.1 mg/kg/infusion dose. One day and 24 days after the last nicotine self-administration session, mice were placed back into the operant chamber and allowed to lever press while experiencing the same sensory cues as during nicotine self-adminstration. However, only the cue light (visual cue) and sound of the nicotine pump (auditory cue) were earned after five active lever presses, in the absence of any nicotine infusions. Following the Day 1 incubation of craving assessment, catheter integrity was tested with the short-acting barbiturate anesthetic Brevital (methohexital sodium, Eli Lilly) to ensure catheters maintained functionality throughout the self-administration period and mice were properly receiving nicotine infusions.

Statistical Analyses

Given that these studies sought to investigate the effects of drug exposure relative to the control condition within each sex, statistical comparisons were performed separately for males and females based on this a priori hypothesis. Data were analyzed by a t-test, one-way or two-way ANOVA with Prism 9 software (GraphPad), as appropriate. Data obtained across sessions was analyzed with a repeated measures two-way ANOVA. Significant main or interaction effects were followed by Bonferroni post-hoc comparison with correction for multiple comparisons. The criterion for significance was set at $\alpha = 0.05$ two-tailed.

RESULTS

Male Adolescent Drug Exposure Alters Body Weight and Reward-Related Behaviors

In these studies, we sought to examine how adolescent exposure to nicotine, THC, or co-exposure may alter later reward- and relapse-related behaviors. For translational relevance to youth, THC was administered orally as related to edible consumption, and nicotine was administered via e-cigarette aerosol exposure. However, for the nicotine treatment, we aimed to compare to our prior findings with subcutaneous injections [24], so this additional group was included. Given the different routes of nicotine administration, we desired to first validate the respective level of nicotine's metabolite, cotinine, for both methods. Moreover, given other findings in our lab suggesting that THC may alter nicotine metabolism [unpublished data], we also examined cotinine levels in the THC and nicotine co-exposure groups. In males, cotinine levels were found to significantly differ across groups (**Figure 3.1B**) (one-way ANOVA, $F(4,32) = 205.7$, $p < 0.0001$). Higher blood cotinine levels

were found across all nicotine-treated groups compared to the vehicle ($p < 0.0001$ for all comparisons), thereby validating the measure. When comparing among drug-treated groups, co-exposure to nicotine vapor and the higher dose of THC led to lower cotinine levels as compared to nicotine vapor alone ($p = 0.0004$) or co-exposure to nicotine vapor and the lower dose of THC ($p = 0.0001$). This indicates that the high dose of THC did alter nicotine metabolism. Importantly, males exposed to only nicotine, whether via injections or vapor, did not differ in blood cotinine levels, indicating that both of these administration methods resulted in similar levels of nicotine exposure.

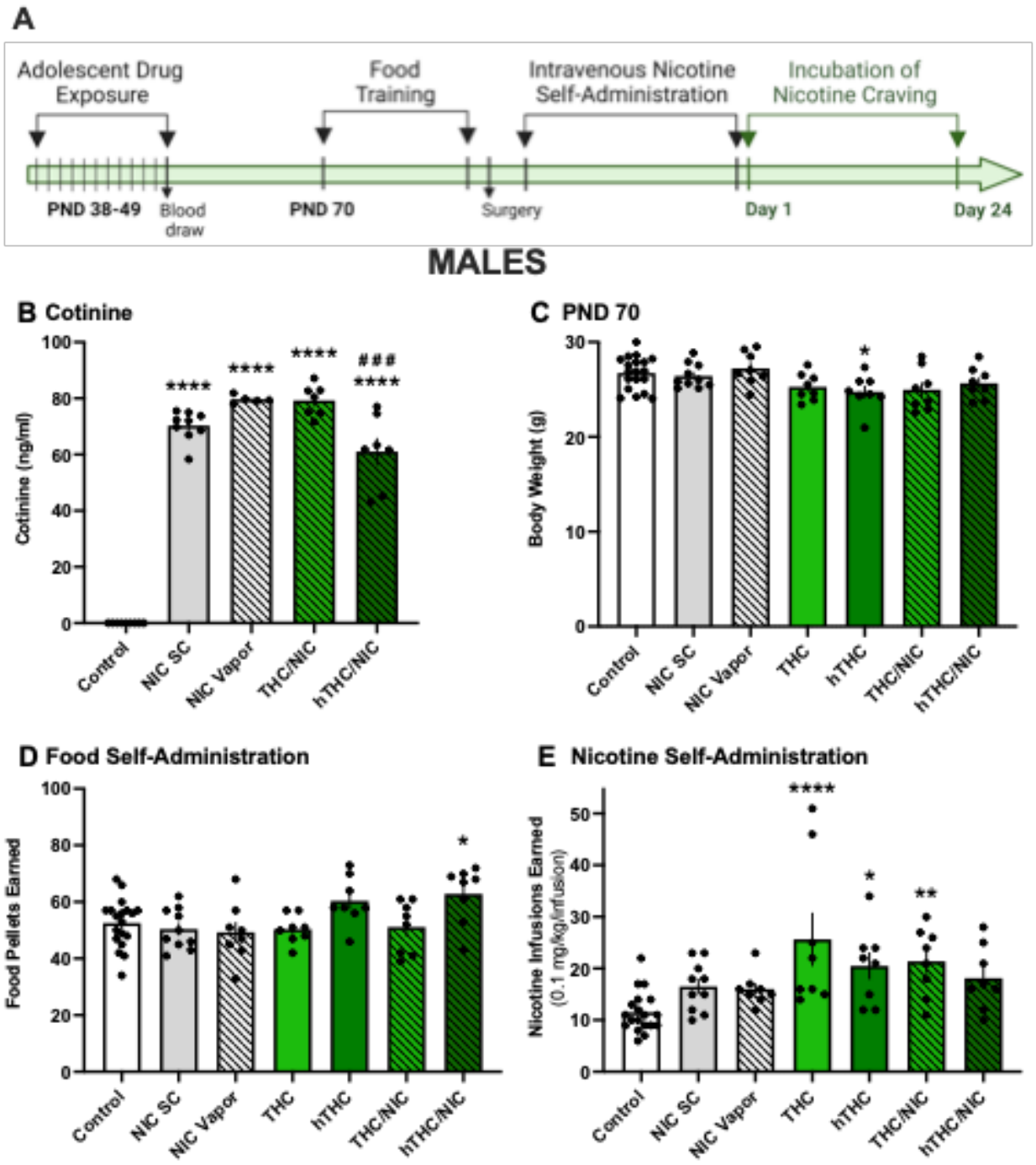


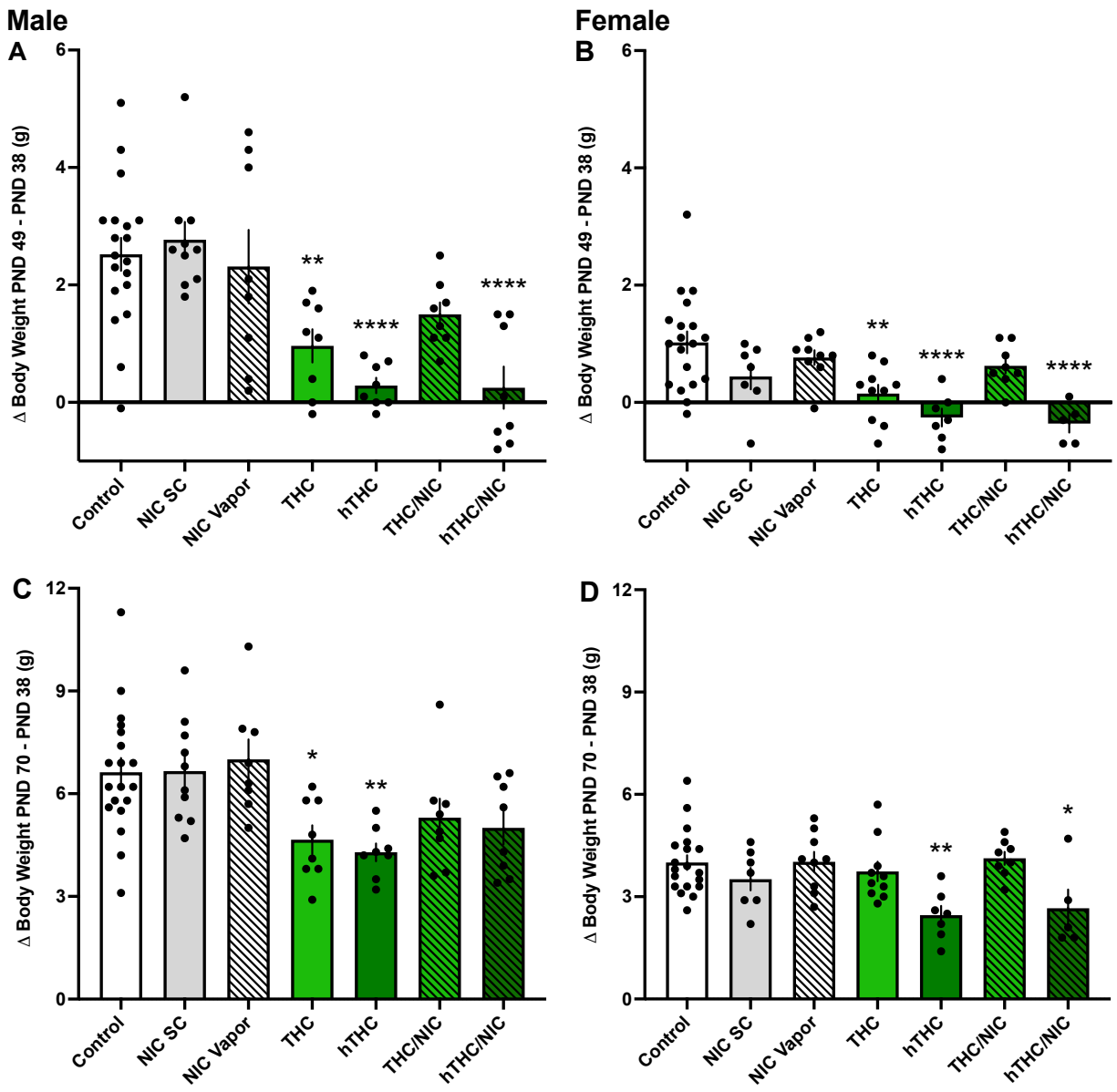
Figure 3.1. Adolescent drug exposure paradigm, differences in cotinine levels, body weight, food rewards and nicotine self-administration in male mice.

(A) Schematic outline of the drug exposure paradigm. Both male and female mice were exposed for 12 consecutive days during adolescence to either vehicle (Control), nicotine via injections (NIC SC) or vapor (NIC Vapor), oral THC at a lower (THC) or higher dose (hTHC), or exposed to both nicotine vapor and THC at a lower (THC/NIC) or higher dose (hTHC/NIC).

After PND 70, all mice completed an operant food training paradigm before undergoing intravenous catheter implantation surgery. Following recovery, they intravenously self-administered nicotine then began an incubation of nicotine craving paradigm. (B) Blood was collected via the facial vein of mice 20 minutes after the last drug exposure session (n=5-9 per group). Male control mice exposed to vehicle vapor had significantly lower cotinine levels in their blood as compared to mice exposed to nicotine, in the presence or absence of THC. ****p < .0001 Control vs. NIC SC, NIC Vapor, THC/NIC, and hTHC/NIC. Interestingly, male mice co-exposed to nicotine vapor and the higher dose of THC had significantly lower cotinine levels as compared to mice exposed to nicotine vapor alone or co-exposed to nicotine vapor and the lower dose of THC. # # #p < .001 hTHC/NIC vs. NIC Vapor, and THC/NIC. Of note, male mice exposed to nicotine either through injection (n=9) or vapor (n=5) did not differ in blood cotinine levels. (C) Male mice (n=8-19 per group) were examined for differences in their body weight at PND 70. Male mice that were adolescently exposed to the higher dose of THC averaged significantly lower body weight than control males. *p < .05 Control vs. hTHC. (D) Male mice (n=8-19 per group) were examined for the ability to learn an operant food training task. Male mice co-exposed to the higher dose of THC and nicotine earned significantly more food pellets than control males. *p < .05 Control vs. hTHC/NIC. (E) Male mice (n=8-19 per group) were assessed on their nicotine self-administration levels in adulthood. Following exposure to either dose of THC or co-exposure to nicotine and the lower dose of THC during adolescence, adult male mice demonstrated an increase in nicotine intake compared with control mice. *p < .05 Control vs. hTHC. **p < .01 Control vs. THC/NIC. ****p < .0001 Control vs THC. Data represent mean values ± standard error of the mean (SEM). PND = Postnatal Day.

Next, since adolescent drug exposure could possibly alter general growth, body weight was examined across the adolescent treatment days and in adulthood. Body weight was measured throughout the treatment period (PND 38-49) and in adulthood (PND 70+). At the beginning of the study (PND 38), none of the male groups differed in weight (Control: 20.11 grams mean ± 0.38 SEM; Nicotine injections: 19.78 ± 0.44; Nicotine Vapor: 20.23 ± 0.43; 5 mg/kg THC: 20.64 ± 0.31; 10 mg/kg THC: 20.5 ± 0.44; 5 mg/kg THC and Nicotine Vapor: 19.66 ± 0.42; 10 mg/kg THC and Nicotine Vapor: 20.66 ± 0.63; one-way ANOVA, $F(6,62) = 0.6782$, $p = 0.6677$). However, body weight differentially changed throughout the adolescent drug exposure period, even though all subjects were provided water and food ad libitum

(**Supplementary Figure 3.1A**) (one-way ANOVA, $F(6,62) = 9.290$, $p < 0.0001$). The post-hoc analysis revealed that males exposed to either the lower ($p = 0.0062$) or higher ($p < 0.0001$) dose of THC, as well as those co-exposed to nicotine and the higher dose of THC ($p < 0.0001$), gained less weight from PND 38 to 49, as compared to control subjects. However, this reduced weight gain persisted into adulthood (PND 70) only for males exposed to THC alone at the low or high dose (**Supplementary Figure 3.1C**) (one-way ANOVA, $F(6,62) = 4.872$, $p = 0.0004$; Post-hoc, Control vs 5 mg/kg THC $p = 0.0175$, Control vs. 10 mg/kg THC $p = 0.0032$). Thus, we next assessed whether the groups differed at the start of the behavioral studies in adulthood (PND 70) (**Figure 3.1C**) (one-way ANOVA, $F(6,62) = 2.848$, $p = 0.0163$). The post-hoc analysis revealed that adolescent exposure to the higher dose of THC led to lower body weight than vehicle exposure ($p = 0.0428$). Thus, these data indicate that adolescent exposure to a higher dose of THC, but not when co-administered with nicotine, induced persistent changes in body growth into adulthood.

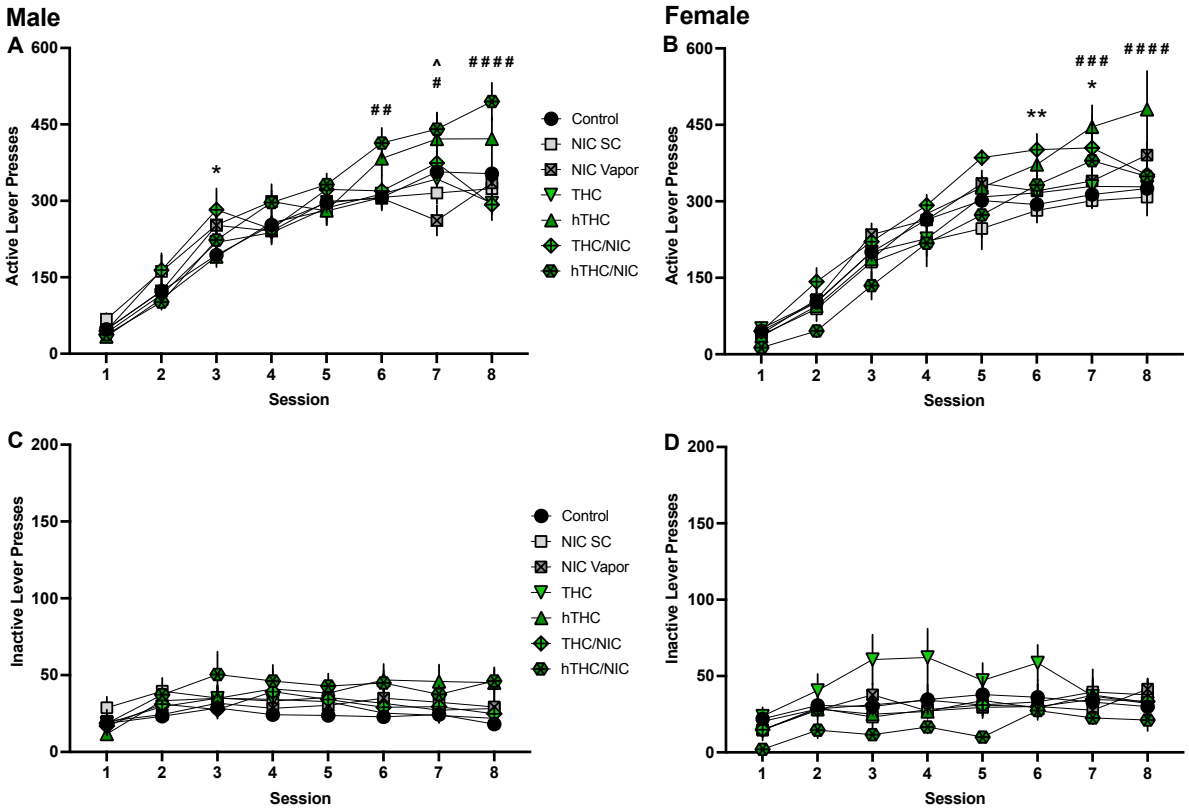


Supplementary Figure 3.1 Changes in body weight over time.
(A) Changes in the body weight of male mice (n=8-19 per group) from postnatal day (PND) 38 to 49 were examined. This time frame is the adolescent drug exposure period. Male mice exposed to either dose of THC or co-exposed to the higher dose of THC and nicotine during adolescence exhibited a smaller change in body weight from PND 38 to 49 as compared to control males. **p < .01 Control vs. THC. ****p < .0001 Control vs. hTHC, and hTHC/NIC. (B) Female mice (n=5-19 per group) also had statistically significant differences in body weight across the adolescent drug exposure period. Specifically, female mice exposed to either dose of THC or co-exposed to the higher dose of THC and nicotine had significantly smaller changes in body weight than control females. **p < .01 Control vs. THC; ****p < .0001 Control vs hTHC, and hTHC/NIC. (C) Changes in body weight prior to drug exposure (PND 38) into

adulthood (PND 70) were assessed. In males (n=8-19 per group), exposure to either dose of THC resulted in smaller changes in body weight from PND 38 to 70 as compared to control subjects. *p < .05 Control vs. THC. **p < .01 Control vs. hTHC. (D) In females (n=5-19 per group), exposure to the higher dose of THC, in the presence or absence of nicotine, resulted in smaller changes in body weight from PND 38 to 70. *p < .05 Control vs. hTHC/NIC. **p < .01 Control vs. hTHC.

To examine whether adolescent drug exposure altered the subjects' ability to learn an operant task, groups were examined for their ability to press a lever to earn food reward. Male subjects were permitted to food train across 8 consecutive sessions, and all groups exhibited a dissociation between the active and inactive levers with training, and further achieved the lever pressing criteria for the fixed-ratio 5 level of reinforcement within a similar number of sessions (one-way ANOVA, $F(6,62) = 0.4506$, $p = 0.8418$). However, some groups differed in the level of maintained lever pressing on the active lever after initially demonstrating proficiency in learning the task (**Supplementary Figure 3.2A**) (repeated measures two-way ANOVA, Treatment group: $F(6,62) = 1.320$, $p = 0.2617$; Session: $F(7,434) = 256.7$, $p < 0.0001$; Interaction: $F(42,434) = 4.001$, $p < 0.0001$). Post-hoc analysis revealed that co-exposure of nicotine and lower dose of THC in adolescence led to a higher level of active lever pressing than the control group in adulthood, but only in session 3 ($p = 0.0291$). In session 7, males exposed to nicotine vape alone exhibited a lower level of active lever presses than the control ($p = 0.0150$). With regard to subjects co-exposed to nicotine and the higher dose of THC, a higher level of active lever pressing was found for sessions 6 ($p = 0.0046$), 7 ($p = 0.0446$) and 8 ($p <0.0001$). To assess whether these differences reflected an overall increase in activity, inactive lever presses were also examined across sessions, but statistically significant differences were not found with the main treatment nor interaction effects (**Supplementary Figure 3.2C**) (repeated measures two-way ANOVA, Treatment

group: F(6,62) = 1.631, p = 0.1537; Session: F(7,434) = 8.420, p < 0.0001; Interaction: F(42,434) = 1.257, p = 0.1367). Thus, to further investigate if the active lever differences are reflected in the number of food pellets obtained across sessions 6-8, we next compared the mean pellets earned. Of note, differences were not found among groups (**Figure 3.1D**) (one-way ANOVA, F(6,62) = 3.368, p = 0.0061), with the exception of the co-exposure nicotine and higher dose THC group that earned significantly more food pellets (p = 0.0298) compared to the control. Therefore, these findings indicate that higher dose THC and nicotine co-exposure during adolescence in males induces more persistent effects on the drive to obtain food in the operant paradigm in adulthood.



Supplementary Figure 3.2 Differences in lever pressing during operant food training. (A) Male mice differed in the average number of lever presses during the operant food training paradigm dependent on group (n=8-19 per group). In session 3, male mice co-

exposed to nicotine and the lower dose of THC had significantly more active lever presses than control subjects. *p < .05 Control vs. THC/NIC. Male mice co-exposed to higher dose of THC and nicotine during adolescence averaged significantly more active lever presses across later food training sessions than control mice. #p < .05 Control vs. hTHC/NIC; # #p < .01 Control vs. hTHC/NIC; # # # #p < .0001 Control vs. hTHC/NIC. In session 7, males exposed to nicotine vapor in adolescence had significantly fewer lever presses than control males. ^ p < .05 Control vs. NIC Vapor. (B) Females (n=5-19 per group) also exhibited statistically significant differences in active lever pressing behavior during food training. In later food training sessions, female mice exposed to higher dose of THC alone or co-exposed to nicotine and the lower dose of THC averaged significantly more active lever presses than control females. *p < .05 Control vs. THC/NIC; **p < .01 Control vs. THC/NIC. # # #p < .001 Control vs. hTHC; # # # #p < .0001 Control vs. hTHC. (C and D) The number of inactive lever presses across food training sessions did not significantly differ among groups in either males (n=8-19 per group) or females (n=5-19 per group).

Next, to determine whether adolescent nicotine and/or THC exposure alters the reinforcing properties of nicotine in adulthood, mice were assessed for intravenous nicotine self-administration. Interestingly, adult males significantly differed in the average number of nicotine infusions earned dependent on adolescent drug exposure (**Figure 3.1E**) (one-way ANOVA, $F(6,62) = 4.895$, $p = 0.0004$). Specifically, compared to control subjects, significantly more nicotine infusions were earned following adolescent exposure to the lower and higher dose of THC ($p < .0001$ and $p = .0187$, respectively), and co-exposure to nicotine and the lower dose of THC ($p = .0079$). Given these findings with THC altering later nicotine intake, it is surprising to note that differences were not found with co-exposure to nicotine and the higher dose of THC, even though this group exhibited a greater drive to obtain food reward. Taken together, these results indicate that adolescent use of cannabinoids have a persistent effect on reward consumption, which is dependent on THC dose, nicotine co-exposure, and type of reward.

Female Adolescent Drug Exposure Alters Body Weight and Reward-Related Behaviors

Since drugs of abuse may differentially alter development dependent on sex, we next examined whether adolescent nicotine, THC, or co-exposure in females results in similar physiological and behavioral outcomes in adulthood. We first examined whether the route of nicotine exposure results in similar blood cotinine, and whether this is affected by THC co-exposure (**Figure 3.2A**) (one-way ANOVA, $F(4,34) = 105.8$, $p < 0.0001$). As above, all of the nicotine treatment groups resulted in a significant level of detectable cotinine (Post-hoc, $p < .0001$ for all comparisons). When comparing among treatment conditions, injections of nicotine resulted in lower cotinine levels than nicotine vapor ($p = 0.0441$) and co-exposure of nicotine and low dose THC ($p = 0.0284$), although it is important to note that all of these groups exhibited levels of cotinine >50 ng/ml which is in the range of that found with human e-cigarette and tobacco smokers [25, 26]. Interestingly, similar to that observed in males, females exhibited significantly lower cotinine levels with nicotine vapor and the higher dose of THC as compared to nicotine vapor alone ($p = .0001$) or co-exposure to nicotine vapor and the lower dose of THC ($p < .0001$), suggesting that the high dose of THC interacts with nicotine metabolism. With regard to body weight, all female groups exhibited similar weights at PND 38 at the time group assignment (Control: 17.03 grams mean ± 0.25 SEM; Nicotine injection: 17.03 ± 0.4; Nicotine Vapor: 17.67 ± 0.34; 5 mg/kg THC: 17.12 ± 0.19; 10 mg/kg THC: 17.13 ± 0.52; 5 mg/kg THC and Nicotine Vapor: 16.93 ± 0.23; 10 mg/kg THC and Nicotine Vapor: 17.25 ± 0.73; one-way ANOVA, $F(6, 58) = 0.4807$, $p = 0.8200$). However, following adolescent drug exposure, females groups did exhibit a difference in their body weights (**Supplementary Figure 3.1B**) (one-way ANOVA, $F(6,58) = 7.370$, $p < 0.0001$). Post-hoc analyses revealed that the females gained less weight from PND 38 to 49 if exposed to

either the lower or higher dose of THC ($p = 0.0018$ and $p < 0.0001$, respectively) or co-exposed to nicotine and the higher dose of THC ($p < 0.0001$), compared to vehicle. We then examined whether these changes persisted into adulthood comparing from PND 38 to PND 70 (**Supplementary Figure 3.1D**) (one-way ANOVA, $F(6,58) = 4.374$, $p = 0.0010$) The post-hoc test revealed that the high dose of THC, either in the absence ($p = 0.0013$) or presence of nicotine ($p = 0.0212$), led to decreased body weight differences that were maintained into adulthood, compared to vehicle. However, at PND70, although a main statistically significant difference was found (**Figure 3.2B**) (one-way ANOVA, $F(6,58) = 2.699$, $p = 0.0222$), the post-hoc analysis did not reveal differences among groups. Even so, a trend was noted with higher dose of THC potentially resulting in a lower body weight than vehicle ($p = 0.0517$). Together, these data indicate that a higher dose of THC during adolescence may have persistent developmental effects on body growth.

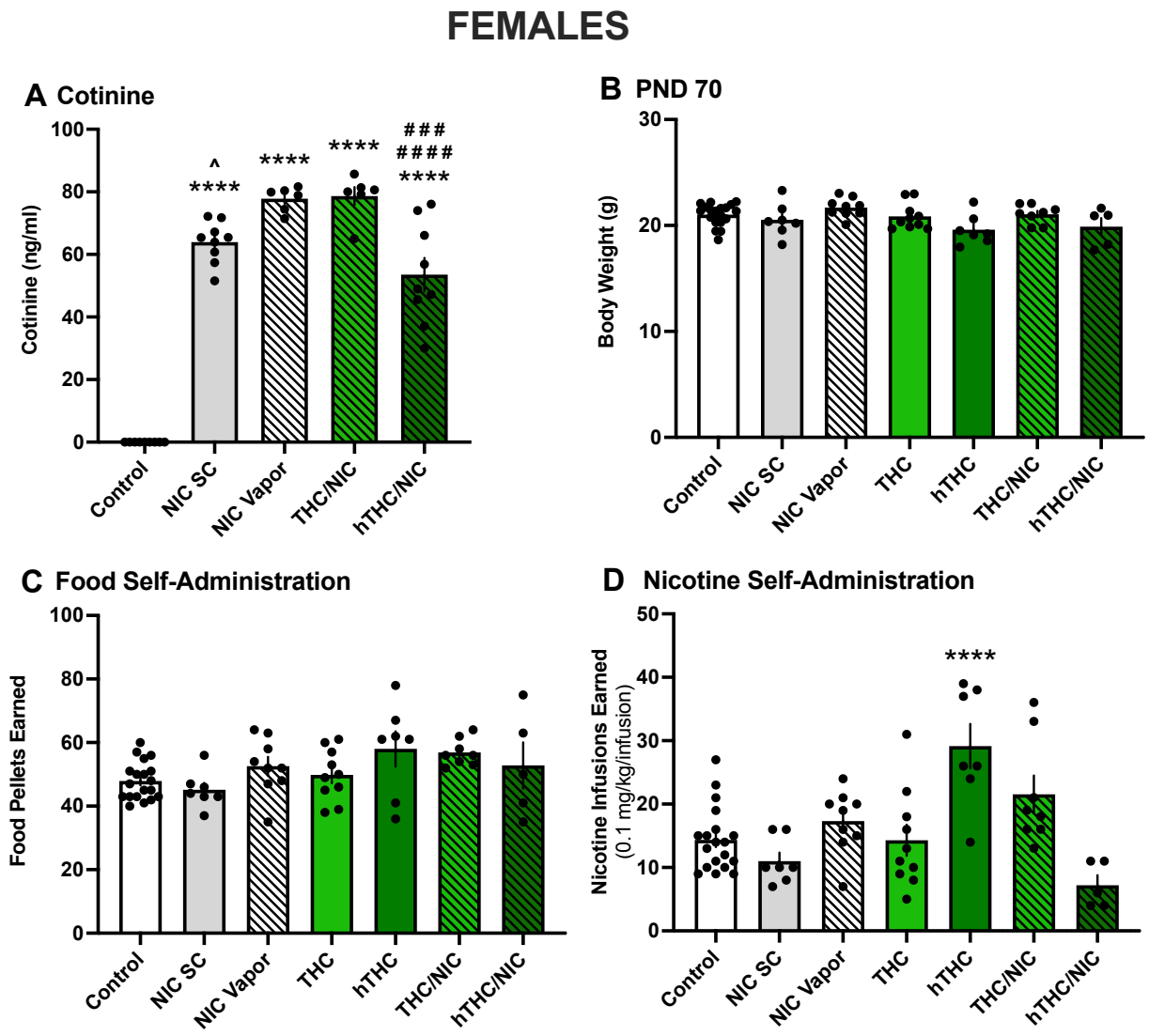


Figure 3.2 Differences in cotinine levels, body weight, food rewards and nicotine self-administration in female mice.

(A) Blood was collected via the facial vein of mice 20 minutes after the last drug exposure session (n=6-9 per group). Female control mice had significantly lower cotinine levels in their blood as compared to mice exposed to nicotine, in the presence or absence of THC. ****p < .0001 Control vs. NIC SC, NIC Vapor, THC/NIC, and hTHC/NIC. Interestingly, female mice co-exposed to nicotine vapor and the higher dose of THC had significantly lower cotinine levels as compared to mice exposed to nicotine vapor alone or co-exposed to nicotine vapor and the lower dose of THC. # # #p < .001 hTHC/NIC vs. NIC Vapor. # # # #p < .0001 hTHC/NIC vs. THC/NIC. Furthermore, female mice exposed to nicotine via injections had significantly lower cotinine levels than those exposed to nicotine via vapor and those co-exposed to nicotine vapor and the lower dose of THC. ^p < .05 NIC SC vs. NIC Vapor, and THC/NIC. (B) Body weight of female mice (n=5-19 per group) was examined at PND 70. Post-

hoc analysis indicated no statistically significant differences in body weight among groups. (C) Female mice (n=5-19 per group) were examined for the ability to learn an operant food training task. Post-hoc analyses revealed no statistically significant differences among groups in the number of food pellets earned across sessions. (D) Female mice (n=5-19 per group) were assessed on their nicotine self-administration levels in adulthood. Following exposure to a higher dose of THC during adolescence, adult female mice demonstrated an increase in nicotine intake compared to control females. ****p < .0001 Control vs. hTHC. Data represent mean values ± SEM.

We next focused our investigations of operant food training in the female mice. Similar to male subjects, females groups exhibited a dissociation between the active and inactive levers with training, and further achieved the lever pressing criteria for the fixed-ratio 5 level of reinforcement within a similar number of sessions (one-way ANOVA, $F(6,58) = 2.096$, $p = 0.0674$). When comparing the number of active lever presses across the 8 training sessions, differences were found among groups, but only after subjects exhibited proficiency in learning the task (**Supplementary Figure 3.2B**) (repeated measures two-way ANOVA, Treatment group: $F(6,58) = 2.065$, $p = 0.0714$; Session: $F(7,406) = 248.1$, $p < 0.0001$; Interaction: $F(42,406) = 2.191$, $p < 0.0001$). The post-hoc analysis revealed that females exposed to the higher dose of THC exhibited a higher level of active lever pressing for sessions 7 ($p = 0.0006$) and 8 ($p < 0.0001$) compared to control. Further, co-exposure to nicotine and the lower dose of THC resulted in greater active lever pressing across sessions 6 ($p = 0.0056$) and 7 ($p = 0.0276$), but no difference on the final session 8 compared to the control. With regard to the inactive lever, significant differences were not found for the main treatment group or interaction effects (**Supplementary Figure 3.2D**) (repeated measures two-way ANOVA, Treatment group: $F(6,58) = 1.074$, $p = 0.3887$; Session: $F(7,406) = 5.729$, $p < 0.0001$; Interaction: $F(42,406) = 1.048$, $p = 0.3951$). Finally, the mean number of food

pellets were examined across session 6-8. No statistically significant differences were found in the post-hoc analysis (**Figure 3.2C**) (one-way ANOVA, $F(6,58) = 2.338$, $p = 0.0433$), but a potential trend was noted with the high dose THC group earning more food pellets than the control ($p = 0.0656$). Thus, these findings indicate that regardless of adolescent exposure, females were able to acquire the food training task, although high dose THC may have led to increased responding to obtain food pellets in later sessions, an effect not found with nicotine co-exposure. We then examined intravenous nicotine self-administration during adulthood in female subjects with a history of drug exposure. Interestingly, statistically significant differences were found in nicotine intake (**Figure 3.2D**) (one-way ANOVA, $F(6,58) = 8.705$, $p < 0.0001$). The post-hoc analysis revealed that a higher dose of THC during adolescence led to increased nicotine intake in adulthood compared to vehicle ($p < .0001$). While not statistically significant, we also noted a trend with the co-exposure nicotine and lower dose of THC group having higher intake compared to control ($p = 0.0539$). Taken together, these findings reveal that a high dose of THC during adolescence increases the drive to consume both food and nicotine in adulthood, an effect which appears to have been counteracted by the co-exposure of nicotine.

Incubation of Nicotine Craving in Males and Females

Re-exposure to the auditory, visual, and/or olfactory cues associated with drug-taking has been shown to enhance relapse-related behaviors [18, 27]. Thus, after intravenous nicotine self-administration acquisition, we examined lever pressing behavior for a visual and auditory cue in the absence of nicotine infusions. Since this procedure has

been mainly used in rats, it was important to first demonstrate that control subjects could exhibit a robust incubation of nicotine craving effect, as validation of this protocol in mice. For our analysis, we also included comparisons of active lever pressing that correspond to the nicotine self-administration data presented in Figures 1E (males) and 2D (females). These data were important to include to determine whether the mice exhibited an extinction burst on the first day of incubation testing (e.g., lever pressing in the absence of nicotine), which could have implications for interpretation of the later incubation effect on Day 24. Therefore, the post-hoc analysis compared incubation day 1 to the other sessions (e.g., Nicotine or Day 24). Consistent with our prediction, subjects exposed to vehicle during adolescence exhibited a significant increase in active lever presses comparing Day 1 to Day 24 of nicotine abstinence; this effect was evidenced in both males (**Figure 3.3A**) (repeated measures one-way ANOVA, $F(2,36) = 28.22$, $p < 0.0001$) and females (**Figure 3.3B**) ($F(2,36) = 19.09$, $p < 0.0001$). In the post-hoc analysis, there was a significant increase in active lever pressing comparing incubation Day 1 to Day 24 for both males ($p < 0.0001$) and females ($p < 0.0001$). However, active lever pressing did not differ when comparing responding for nicotine infusions to incubation Day 1 (no nicotine). Thus, these findings demonstrate that incubation of nicotine craving can be readily detected in mice.

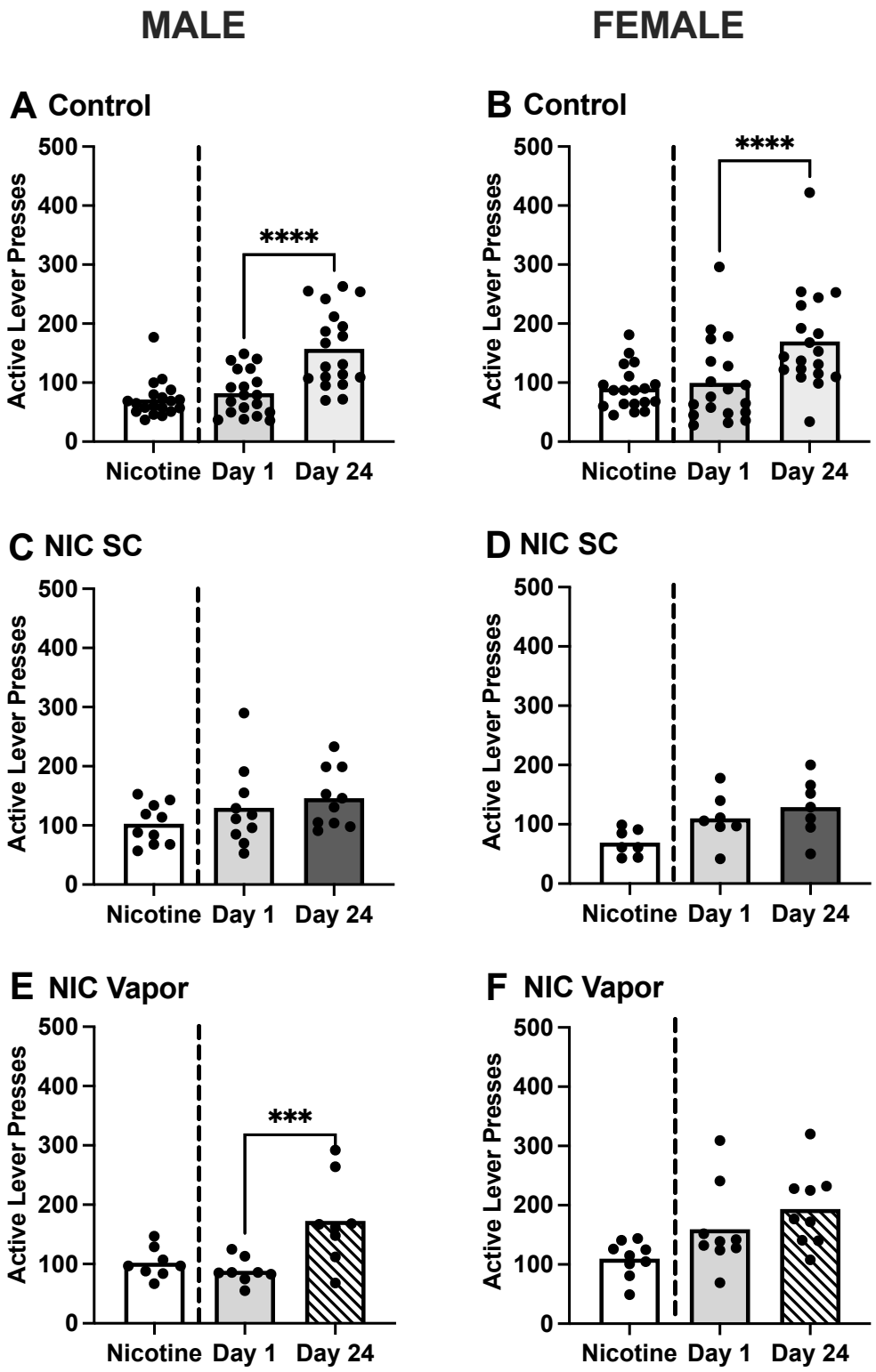


Figure 3.3 Adulthood incubation of nicotine craving following adolescent exposure to vehicle, nicotine via injections, or nicotine vapor

(A and B) Male and female mice (n=19 per group) not exposed to any drugs during adolescence exhibit an incubation of nicotine craving, in which drug-seeking behavior in the form of lever pressing significantly increases from incubation day 1 to day 24. ****p < .0001 Male Control Day 1 vs. Day 24; ****p < .0001 Female Control Day 1 vs. Day 24. (C and D) Interestingly, male and female mice (n=7-10 per group) exposed to nicotine via injections during adolescence do not demonstrate a significant increase in active lever pressing from Day 1 to Day 24 during the incubation period. (E and F) Male mice (n=8) exposed to nicotine vapor during adolescence do demonstrate this enhanced drug-seeking following incubation; however, female mice (n=9) also adolescently exposed to vaporized nicotine do not. ***p < .001 Male NIC Vapor Day 1 vs. Day 24. Data represent mean values ± standard error of the mean (SEM).

Adolescent Nicotine Exposure

We then examined the effects of adolescent drug exposure with the incubation of craving paradigm. Interestingly, males exposed to nicotine injections did not exhibit an incubation of craving effect (**Figure 3.3C**) (repeated measures one-way ANOVA, $F(2,18) = 3.055$, $p = 0.0721$), whereas males exposure to nicotine vapor exhibited incubation of craving (**Figure 3.3E**) (repeated measures one-way ANOVA, $F(2,14) = 12.35$, $p = 0.0008$). For the nicotine vapor group, the post-hoc analysis revealed an increase in active lever pressing on Day 24, as compared to Day 1, of incubation ($p = 0.0008$), but no differences were found comparing Nicotine to Day 1. This effect was interesting given that these groups did not differ in the level of cotinine, suggesting that the differences in duration of daily adolescent exposure (acute injection vs. 1 hr inhalation of aerosol) may be relevant. In contrast, female mice exposed to nicotine did not exhibit an incubation of craving effect based on adolescent history of nicotine injection (**Figure 3.3D**) (repeated measures one-way ANOVA, $F(2,12) = 4.553$, $p = 0.0338$; Post-hoc, $p > 0.05$ for all comparisons) or nicotine vapor exposure (**Figure 3.3F**) (repeated measures one-way ANOVA, $F(2,16) = 8.003$, $p = 0.0039$; Post-hoc, $p > 0.05$ for all comparisons). While both of the ANOVAs indicated a statistically significant effect,

post-hoc analyses did not reveal significant differences among sessions, although with nicotine vapor exposure a trend was noted between the Nicotine and Day 1 sessions ($p = 0.0563$) suggesting a potential burst in responding. Given concerns regarding the Nicotine session interacting with data analysis based on multiple comparisons, we also conducted individual t-tests comparing incubation Day 1 and Day 24 but no differences were found (Nicotine injections, $t(6) = 0.8893$, $p = 0.4081$; Nicotine vapor, $t(8) = 1.335$, $p = 0.2188$), thereby supporting the initial findings for a lack of incubation in females following adolescent nicotine exposure.

Adolescent THC Exposure

Given the differences found in body weight and food training with some of the THC exposure groups, we predicted that significant differences would also be found for incubation of craving. Adolescent exposure to the lower dose of THC did not interfere with incubation of craving effect in males, as increased responding was found comparing Day 24 to Day 1 (**Figure 3.4A**) (repeated measures one-way ANOVA, $F(2,14) = 9.101$, $p = 0.0029$; Post-hoc, Day 1 vs. Day 24 $p = 0.0033$). This effect was also found with male adolescent exposure to the higher dose of THC (**Figure 3.4C**) (repeated measures one-way ANOVA, $F(2,14) = 5.244$, $p = 0.0200$; Day 1 vs. Day 24 $p = 0.0345$). For the females, a similar finding occurred at the lower dose of THC, in which there was an increase in active lever pressing on Day 24, as compared to Day 1, of incubation (**Figure 3.4B**) ($F(2,18) = 4.637$, $p = 0.0237$; Day 1 vs. Day 24 $p = 0.0357$). Interestingly, a significant effect was found at the higher dose of THC (**Figure 3.4D**) (repeated measures one-way ANOVA, $F(2,12) = 11.000$, $p = 0.0019$) in

which females demonstrated an increase in active lever pressing on incubation Day 1 compared to baseline Nicotine ($p = 0.0082$), but a further incubation of craving effect was not found comparing Day 24 to Day 1. Of note, this was the only condition in which a significant difference was found between the Nicotine and Day 1 sessions. These findings suggest that the higher dose of THC during adolescence may have led to overall increased active lever pressing, suggesting either overall increased general activity (consistent with the higher level of responding for food training, e.g., see Supplementary Figure 2B), or alternatively, a premature incubation effect with higher immediate and persistent drug seeking behavior.

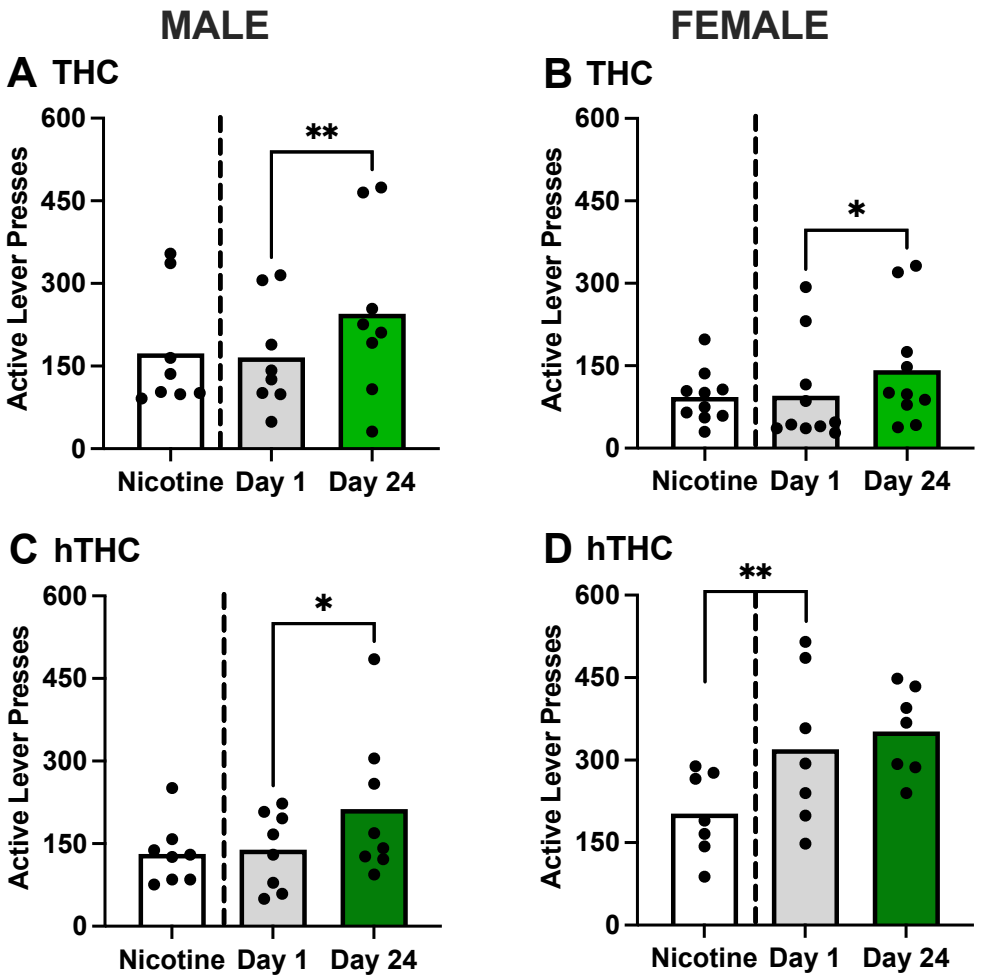


Figure 3.4 THC dose-dependent differences in adulthood nicotine-seeking
(A and B) Both male (n=8) and female (n=10) mice exposed to a lower dose of THC during adolescence do exhibit an incubation of nicotine craving effect in adulthood. **p < .01 Male THC Day 1 vs. Day 24. *p < .05 Female THC Day 1 vs. Day 24. (C) Additionally, males exposed to the higher dose of THC (n=8) also exhibit this increased lever pressing behavior following a 24-day abstinence. *p < .05 Male hTHC Day 1 vs. Day 24. (D) However, female mice (n=7) exposed to the higher dose of THC during adolescence do not demonstrate a significant increase in active lever pressing on day 24 as compared to day 1 of the incubation period. But these subjects do have a significant increase on day 1 as compared to the average lever presses during nicotine exposure. **p < .01 Female hTHC Nicotine vs. Day 1. Data represent mean values ± standard error of the mean (SEM).

Co-exposure of Nicotine and THC

Finally, we sought to determine whether nicotine and THC together would have unique effects on relapse-related behaviors. Surprisingly, co-exposure elicited differential outcomes compared to what we previously reported for single drug exposure. Specifically, adolescent males co-exposed to nicotine and THC exhibited a robust incubation of craving effect for both the lower dose THC (**Figure 3.5A**) (repeated measures one-way ANOVA, $F(2,14) = 11.090$, $p = 0.0013$; Post-hoc, Day 1 vs. Day 24 $p = 0.0016$) and higher dose THC (**Figure 3.5C**) (repeated measures one-way ANOVA, $F(2,14) = 18.89$, $p = 0.0001$; Post-hoc, Day 1 vs. Day 24 $p = 0.0022$). Similarly, adolescent females co-exposed to nicotine and THC demonstrated significant incubation effects for both the lower dose THC (**Figure 3.5B**) ($F(2,14) = 11.39$, $p = 0.0012$; Post-hoc, Day 1 vs. Day 24 $p = 0.0018$) and higher dose THC (**Figure 3.5D**) ($F(2,8) = 21.98$, $p = 0.0006$; Post-hoc, Day 1 vs. Day 24 $p = 0.0017$). For all of the above co-exposure comparisons, statistically significant differences were not found between baseline Nicotine and incubation Day 1. Together, these findings indicate that nicotine and THC can interact to induce a differential effect than either substance alone

during development, thereby sustaining a heightened response to drug associated cues to propagate increased nicotine seeking behavior and potential risk of relapse.

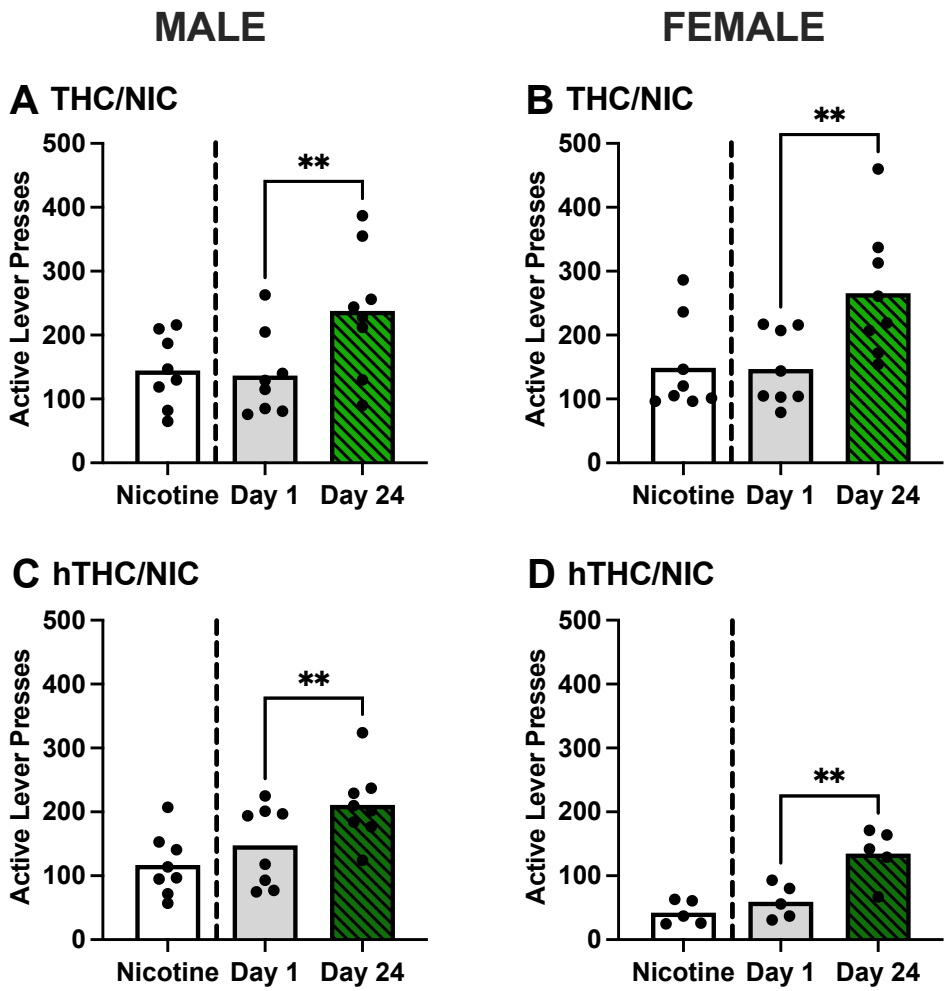


Figure 3.5 Adolescent co-exposure to vaporized nicotine and different doses of THC does not alter incubation of nicotine craving in adulthood
(A and B) Male and female mice (n=8 per group) co-exposed to nicotine vapor and a lower dose of THC during adolescence do demonstrate an incubation of nicotine craving effect in adulthood. **p < .01 Male THC/NIC Day 1 vs. Day 24; **p < .01 Female THC/NIC Day 1 vs. Day 24. (C and D) Additionally, male and female mice (n=5-8 per group) exposed to nicotine vapor and the higher dose of THC during adolescence do also demonstrate this significant increase in active lever pressing following the incubation period. **p < .01 Male hTHC/NIC Day 1 vs. Day 24; **p < .01 Female hTHC/NIC Day 1 vs. Day 24. Data represent mean values ± standard error of the mean (SEM).

DISCUSSION

This study sought to determine whether prior nicotine and/or THC exposure during adolescence would alter operant learning, drug reinforcement, and nicotine seeking behaviors. Importantly, we found that nicotine exposure in adolescence regardless of route of administration resulted in significantly high levels of cotinine in both sexes; but co-exposure with the higher dose of THC altered the metabolism of nicotine as evidenced by significantly lower cotinine levels in these subjects than those exposed to nicotine alone. Males that were co-exposed to nicotine and the higher dose of THC in adolescence also exhibited increased food self-administration in adulthood. In contrast, none of the female groups differed in food self-administration. Furthermore, males that were exposed to either dose of THC alone in adolescence or co-exposed to nicotine and the lower dose of THC had increased nicotine intake in adulthood. Whereas females with adolescent exposure to only the higher dose of THC exhibited increased nicotine intake in adulthood. Following nicotine self-administration, both male and female control mice exhibited increased nicotine-seeking behaviors following a 24-day abstinence period. Males exposed to nicotine vapor or either dose of THC alone also demonstrated this increased cue-induced nicotine seeking. However, adolescent exposure to nicotine via injections in either sex or to nicotine vapor in females did not result in this later enhanced nicotine seeking behavior. Interestingly, for both sexes, co-exposure to nicotine and THC at either dose in adolescence does result in this incubation of nicotine craving effect in adulthood, even when single drug exposure does not.

Impact of Adolescent Drug Exposure on Operant Learning and Nicotine Intake

Nicotine and cannabis use during adolescence has been shown to have lasting implications on later learning and memory [28, 29]. However, our findings did not reveal any differences in the subjects' abilities to learn the operant food training task in either sex. All groups were able to sufficiently dissociate between the active and inactive levers during training and further achieved the lever pressing criteria within a similar number of sessions. Rather, differences in lever pressing behavior were only found in later sessions once the learning already occurred. Males co-exposed to the higher dose THC and nicotine during adolescence as well as females exposed to the higher dose of THC alone demonstrated a higher level of responding for this task and maintained a more persistent drive to obtain food, which may be indicative of greater hedonic value of food for these subjects.

Following this operant learning paradigm, we wanted to assess how adolescent drug exposure might impact nicotine reinforcement in adulthood. In our previous studies we have found that exposure to the synthetic cannabinoid WIN 55,212-2 (WIN) and co-exposure to nicotine and WIN together in adolescence increases subsequent adulthood intake of a low dose of nicotine in males, but decreases intake of low and moderate nicotine doses in females [30]. Our current experiments revealed that females exposed to the higher dose of THC in adolescence self-administered more nicotine in adulthood at a moderate nicotine dose than control subjects. Males exposed to either the lower or higher dose of THC or co-exposed to nicotine and the lower dose of THC also self-administered more nicotine at this dose. These findings differ compared to our previous studies where we did not see any differences in nicotine intake at this dose following adolescent cannabinoid drug exposure in males and

reduced nicotine intake in females [30]. But given that the method of drug exposure, oral as compared to previously injected, and the drug itself, THC compared to WIN, are changed, these differences are not too surprising. Rather, it further adds to the complexity of this story when parsing out the effects of adolescent drug exposure on later drug-taking behaviors. In humans, women with a history of cannabis use are four times more likely to become regular cigarette smokers and almost three times as likely to develop nicotine dependence which is supported by our finding of increased nicotine intake in adult female mice who were previously exposed to THC [31]. Additionally, men with a history of cannabis use are also more likely to become daily cigarette smokers which aligns with our current findings as well [32].

Of note, it was unexpected that the groups co-exposed to nicotine and the higher dose of THC did not exhibit any differences in nicotine intake. These groups did have the initial differences in cotinine levels which we suspected would alter later drug-taking. While all of the nicotine exposed groups in both sexes had a significant level of cotinine in their blood, both males and females co-exposed to the higher dose of THC and nicotine together had lower cotinine levels than those exposed to nicotine vapor alone and those co-exposed to the lower dose of THC and nicotine vapor. This suggests that the higher dose of THC may impact the metabolism of nicotine. In support of these findings, another study confirms that in human smokers, co-users of both nicotine and THC have lower cotinine levels than tobacco only smokers [33]. Yet although males co-exposed to nicotine and the higher dose of THC exhibited a greater drive to obtain food reward that was not reflected in their nicotine intake. Thus, taken together, findings from these experiments demonstrate that in males, THC or nicotine and lower dose THC co-exposure in adolescence, and in females exposure to higher

doses of THC during adolescence have persistent developmental effects and increase the drive to consume both food and nicotine in adulthood.

Adolescent Drug Exposure Alters Later Incubation of Nicotine Craving

Finally, we wanted to assess the impact of this adolescent drug exposure on cue-induced nicotine seeking in adulthood. We were first able to reliably demonstrate the incubation of nicotine craving effect in both male and female control mice. Then we found that chronic adolescent exposure to nicotine or THC differentially alters later incubation of nicotine craving based on route of administration and dose. Specifically, both male and female mice that were exposed to nicotine via injections and females exposed to nicotine vapor during adolescence did not have enhanced nicotine-seeking as adults following an extended withdrawal period. These findings indicates that nicotine-associated cues may not induce craving across abstinence in some subjects that have an adolescent history of nicotine exposure. Surprisingly, males that were exposed to nicotine vapor during adolescence did have the incubation of nicotine craving effect in adulthood. It is important to note that the cotinine levels for males exposed to nicotine alone regardless of route of administration did not differ, which emphasizes that the duration of the daily nicotine exposure (acute injection as compared to one hour inhalation of aerosol) has unique implications on this later drug-seeking behavior. This contention needs to be further explored with more specific studies but the notion is supported with prior findings that duration of nicotine exposure via osmotic minipumps compared to injections at the same dose alters nicotine withdrawal [34].

Furthermore, given the differences in nicotine intake among the male THC exposure groups, we were surprised to find that they maintained the incubation of nicotine craving effect. However, another study found similar results in which adolescent THC exposure in male mice does not alter later stress- and cue-induced reinstatement of nicotine-seeking following extinction [35]. In females, while the lower dose THC exposure group did have an increase in nicotine-seeking on day 24 as compared to day 1 of the incubation, the higher dose THC group did not. Instead, the higher dose THC female subjects exhibited an increase in active lever presses on day 1 as compared to their lever pressing during nicotine self-administration. This finding suggests that the higher dose of THC during adolescence may have led to overall increased active lever pressing, which is consistent with the higher level of responding for food training, or it may be a premature incubation effect with higher immediate and persistent drug seeking behavior.

Importantly, co-exposure to nicotine vapor and THC at either dose does result in the incubation of nicotine craving effect. We expected these results as human adult co-users are twice as likely as tobacco smokers who do not use cannabis to continue smoking tobacco [36]. This could be due to the cannabinoids enhancing the effects of nicotine-associated cues in reinstating the drug-seeking behavior after a quit attempt [37]. Moreover, for the females, although single drug exposure to nicotine or the higher dose of THC alone does not result in the incubation effect, perhaps in these poly-drug exposure conditions the nicotine and cannabinoids interact during development to alter the responsivity of nicotine-associated cues in reinstating drug-seeking behavior. Thus, the adolescent co-exposure to both drugs could result in a heightened response to the drug-associated cues later in life.

Implications of Prior Drug History on Treatment of Nicotine Use Disorder

Patients' drug histories are an important factor for treating substance use disorders. Our prior findings demonstrated that acute and chronic pre-treatment with a synthetic cannabinoid can reduce nicotine intake in male and female mice; however, if either sex had been previously exposed to nicotine or cannabinoids during adolescence, this reduction does not occur [30]. Thus, prior drug history may be a mediating factor in the effectiveness of pharmacological cessation treatments. Furthermore, in another study, the offspring mice of parents who were exposed to nicotine do not demonstrate an incubation of nicotine craving effect [38]. This indicates that the effects of drug exposure persist not only for the later drug-seeking and cue responsive behaviors in one subject, but it could have lasting generational impacts for their offspring as well.

In conclusion, adolescent exposure to nicotine and/or THC does alter operant learning, drug intake, and relapse-related behaviors in adulthood in a sex-dependent manner. Taken together, these results indicate that adolescent use of cannabinoids have a persistent effect on reward consumption, which is dependent on THC dose, nicotine co-exposure, and type of reward. These studies emphasize the impact of prior drug history on later drug-associated behaviors and susceptibility to relapse as well as the importance of being cognizant of adolescent drug use in patient populations when navigating personalized approaches to treating adulthood substance abuse.

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Chapter 4: Increasing Diversity, Enhancing Equity, and Promoting Inclusion in the Field of Neuroscience

INTRODUCTION

Diversity, equity, and inclusion (DEI) have been hot topics in recent years. In particular since the worldwide protests against police brutality and social injustices in 2020, there has been a resurgence in the push for creating more equitable and inclusive environments within the field of neuroscience and academia as a whole. However, true DEI work must go beyond just increasing the number of historically excluded scholars in the laboratories and classrooms; they must also be actively included, given a voice to push for change, and supported in their efforts. My DEI efforts as a graduate student have included serving as an Interdepartmental Neuroscience Program graduate student representative, Competitive Edge peer mentor, and department representative for the Diverse Educational Community and Doctoral Experience program. In each of these roles, I strived to create safe spaces for first-generation, Black, Indigenous, Latinx scholars and women to feel welcomed in neuroscience. I also offered reassurance that they belong in the field, advocated on their behalf, and provided needed support as they navigate academia. In doing so, I went beyond simply recruiting a more diverse cohort into the neuroscience program to help ensure their retention by cultivating a community in which they could thrive.

Perhaps my greatest achievement outside my scientific research has been my efforts to support historically marginalized people in neuroscience and founding the global non-profit, Black In Neuro. Black In Neuro is an international organization that aspires to diversify the neurosciences by building a community that celebrates and empowers Black scholars and professionals in neuroscience-related fields. We also aim to provide professional development resources and increase the visibility of Black neuroscientists to inspire the next generation and dismantle stereotypes of what a neuroscientist can be.

Through offering these services, and access to culturally competent mentorship, it has strengthened the resolve of Black and other historically marginalized trainees and early career scientists all around the world to want to stay in the field.

Through Black In Neuro, we build a community, offer support for the scholars, and also share advice with departments, institutions, and allies on ways to effectively support persons from diverse backgrounds. To this end, I have written and co-authored several commentaries that are highlighted below.

How to Better Support Black Trainees in the Biomedical Sciences

Angeline Dukes. *Nature Medicine* 2020. DOI: 10.1038/s41591-020-1101-3

The relentless violence against Black people takes an overwhelming emotional toll on Black trainees. In those we continue to lose, we see our families, our friends and our own lives being taken.

In June 2020, amidst the worldwide protests about the murders of George Floyd, Breonna Taylor, Ahmaud Arbery and countless others who were the victims of police brutality and white supremacy, I was lost and exhausted. I had been working on advancing to candidacy for my doctoral degree, but I was drowning in the repetitive videos of Black bodies being brutalized. I was struggling to juggle my research with the fear of losing loved ones to COVID-19, a constant fight against microaggressions and stereotypes, and the greater fear of our lives being taken for simply existing while Black.

Since the vast majority of scientific researchers in the USA are non-Black, it can be daunting to express how these events impact my mental health and my ability to be 'productive'. Not wanting to confirm the imposter syndrome that I battle regularly as a first-generation college graduate, I was very hesitant to tell my PI about the unique challenges I was facing. When I finally worked up the courage to talk to her, I was grateful that she was incredibly receptive and offered support in every way possible. But not every Black trainee is so lucky.

As a PI and mentor, your reactions about these events can dictate whether your trainees ever feel safe to speak up again. Before we are scientists, we are people. Our self-advocacy requires an immense amount of bravery. It is consuming, is psychologically draining and could cause future troubles for us. So, not every underrepresented person wants to confront all instances of racism head-on. But with the perpetual anti-Black violence still occurring, I want to give a few suggestions for mentors to better support their Black trainees.

Make it very clear that you are an ally. Don't wait for us to start the conversation, because your silence speaks volumes. Let it be known if your office is a safe space. Host quarterly lab meetings that are focused on efforts the lab is making to become anti-racist and on current issues affecting diverse students. Critically think about the ways in which your research impacts minority and low-income communities. Call out your colleagues on their racism.

Educate yourself. Become aware of the systemic hurdles that every Black student has had to overcome to get to this point. Black Americans hold ~2% of the national wealth, which means less access to private schooling, tutors and prep programs. Standardized tests have been shown to be biased against minority students and those belonging to a lower socioeconomic status. Almost half of Black students enrolled in a postsecondary institution are first-generation college students, which means they may not know about as many scholarship or internship opportunities as their peers do. This isn't even addressing the racial profiling, subpar medical care and over-policing that takes a physiological toll. Take these into consideration when considering graduate school applicants and hiring. Furthermore, actively encourage applicants from nationally funded diversity initiatives that

uplift Black students in the biomedical sciences, such as Maximizing Access to Research Careers, and Diversity Specialized Predoctoral to Postdoctoral Advancement in Neuroscience.

Speak up for your Black trainees. Advocate for us when we're not in the room. Nominate us for awards and speak highly of our efforts. Let us know about fellowships, travel grants and other opportunities that can help advance our careers. Teach us how academia works and the 'unspoken' etiquette in the field.

Use your position of power to be a champion for equality and racial justice. You do not have to belong to an underrepresented group to support the people in that group. Demand that your departments and schools hire diversity and inclusion experts to host implicit-bias workshops and cultural-competency trainings. When they do want to hear from the current Black student and faculty perspective, find ways to compensate them for this diversity work. But also recognize that the one Black student cannot speak for all Black people. Black experiences are not a monolith.

Ensure Black leaders in your field are invited to give research talks at conferences, symposia and departmental seminar series. Encourage everyone to attend, not just the underrepresented students, because perceptions and stereotypes can be changed all around.

If you do not identify as the same gender, race or background as your trainee, help them find a mentor who does. My advisor and I have a mutual understanding of sexism in science. But as a white woman, she cannot fully understand how racism is compounded in my experience. One of the best things she did was to connect me with another Black neuroscientist and professor. Although he is across the country, I appreciate being able to discuss being a Black person in the field without explaining the underlying nuances of my

experiences. Having both of them as mentors can help me navigate multiple intersections of my academic identity.

Recently, I and other neuroscientists worldwide created an initiative with the goals of celebrating Black excellence in neuro-related fields, building community and helping young Black scholars find mentors. At BlackInNeuro.com, we have an ever-expanding list of fellowships and other helpful resources, as well as profiles of Black people in these fields at all levels who are willing to serve as mentors. This is a valuable connection for your Black trainees.

This is not an exhaustive list of all the things you can do. But it is a start. As a Black woman in neuroscience, I can inspire Black students to see themselves in science. I can help guide them to overcome seemingly insurmountable obstacles. But I cannot do it alone. We need non-Black allies to support, encourage and help mentor Black students in the best ways possible. Everyone can do something to make a difference. But don't just do it now when the world is watching — do it always.

An Open Letter to Past, Current and Future Mentors of Black Neuroscientists

Kaela S. Singleton, Rackeb Tesfaye, Elena N. Dominguez & **Angeline J. Dukes**.

Nature Reviews Neuroscience 2020. DOI: 10.1038/s41583-020-00421-9

We as Black trainees in neuroscience and co-founders of Black In Neuro wrote this open letter to thank the phenomenal mentors who came before us. We also aim to encourage and give advice to future mentors on how to effectively mentor the next generation of Black researchers.

Dear Neuroscience Community,

After years of racial injustice, the many recent BlackInX movements have highlighted the experiences of Black trainees in academia [1]. We — as Black trainees — account for only 6% of all neuroscience PhD students in the USA, despite making up 14.7% of the population nationwide. In the UK, Black students account for 4% of graduate research trainees, yet account for only 1.2% of trainees funded by UK research councils [2]. Furthermore, in some other countries, like Canada, the number of Black PhD students regardless of field is not recorded at all.

The work of BlackInX movements have shown that Black trainees are more than data points and more than solutions to diversity, equity and inclusion (DEI) efforts. Yet, we still struggle to feel like more than statistics. We know that neurons and glia need the proper environmental support to develop into mature, unique cells. Like those cells, trainees need the proper support and guidance to succeed in academia. Guidance in the form of mentorship

is a core factor for developing a positive scientific identity, maintaining well-being during graduate school, and achieving academic success and career advancement [3-5]. However, based on collective anecdotes from peers, we know it is rare that Black trainees receive proper mentorship and investment in their development. To this end, we write to thank the mentors who have given us grace and unwavering support in our journeys towards leadership and we ask the future mentors of Black trainees to do the same.

There are varying approaches to mentorship in neuroscience. Some mentors foster your love for the hands-on, problem-solving nature of the field, whereas others train you to think critically about your data and push you to face more challenging questions. Some are more involved in asking questions about your life outside of the lab, while others prefer to keep it strictly science-focused. Despite such differences in mentorship styles, our experiences have taught us that good mentorship relies on these fundamental principles: compassion, advocacy and support. With these values, we see that our mentors believed in the Black In Neuro initiative and in us as individual Black trainees. They believed we would not only do something great but be something great.

To our past and current mentors who have shown us compassion in the face of our failed experiments and personal struggles: you've breathed confidence into us. As we faced obstacles as Black trainees in science and in life, your compassion quieted our insecurities about becoming capable neuroscientists. To those mentors who went above and beyond to embrace the intersections of our identities as Black, Afro-Latinx, Immigrants, LGBTQIA+ and more: you ensured that we had faith in not just in our science but also in ourselves. For the mentors who uprooted the seeds of doubt caused by systematic racism in the field: you gave us a sense of belonging in the neuroscience community.

From those of us who were guided by Black mentors: you taught us the value of representation. Seeing someone who looks like you achieve your dreams sparks ambition, curiosity and hope. It permits us to breathe easier. Your presence increases our sense of belonging in academia and our desire to stay here [6]. As Black scholars in predominantly white spaces, we thrive on these interactions; but we know it comes at a cost to you. You navigate inequitable spaces, receiving fewer grants, authorships and lower salaries [7-10], while continuing to bear the brunt of DEI work to make this a better space for us. We are motivated by your leadership and seek to guide future Black neuroscientists in the same manner.

To present and future mentors of Black trainees: we emphasize that we need mentors to teach us how to navigate academia as individuals who are 'breaking the mold' of what a scientist looks like. We need mentors who are champions, and who, rather than ignoring our identity, celebrate it. We are multifaceted individuals who are often pioneers not just in STEM but in our families as first-generation graduates. We are minority ambassadors who juggle lab work with necessary outreach initiatives. We are mentors to marginalized students because we understand the value of representation. We are consultants on unpaid DEI efforts in the department. Most importantly, we are human beings who are expected to work diligently while witnessing the egregious social injustices faced by Black people worldwide. We need mentors who acknowledge that all of these, often undervalued, duties are born out of necessity, not by choice. We need mentors to teach us how to navigate predominately white spaces and who actively try to diversify them. As Black trainees dealing with micro-aggressions and macro-aggressions daily, we need representation and community.

Although pipeline programs and other diversity initiatives have trained and successfully guided many of us through the neuroscience field, invested mentors truly make a difference. We are brought to the proverbial 'table' through these wonderful initiatives, but we need in-lab support to keep us there. All of the diversity-led funding in the world will not retain a budding scholar who lacks critical guidance. As our mentors, you can provide us with opportunities to publish, present our work, co-author grants, develop networks and teach us to negotiate salaries. Most importantly, as future Black leaders in the field, we need to know that you believe that we belong in the neuroscience community. This means that you will advocate for us, even when we're not in the room. That you will not stay silent when we encounter toxic situations and colleagues.

We have experienced both negative and positive mentorship. Negative mentorship destroyed our confidence and made us question if we can succeed. This self-doubt can dissuade us from staying in the field. It becomes a self-fulfilling prophecy of the 'leaky pipeline' as we seek community elsewhere. Conversely, positive mentorship has often been the deciding factor for our retention in STEM. One good mentor, regardless of ethnicity, gender or socioeconomic status, can make all the difference. Good mentorship gave us confidence not just in our scientific ability but also in our success, our struggles and what is perhaps the most important conviction — that we belong. Thus, our experiences in inclusive training environments with exceptional mentors contribute to our goal of supporting, uplifting and cherishing all Black scientists.

To the Black In Neuro community: we know that many of you lack the mentorship you deserve or have never met another Black neuroscientist. That is why Black In Neuro was created. Founded on the ideals of support and visibility, we welcome you to our family and

encourage you to contact our community members for mentorship, regardless of which academic stage you are in. We want our legacy to live on in future generations of Black neuroscientists. If you are a non-Black mentor of Black trainees, connect your mentees to Black In Neuro events and help them find other mentors through our profile pages. Support your Black trainees in and outside of the lab to cultivate not just their scientific career but also their development into exceptional leaders and mentors.

True diversity, equity and inclusion rely on representation and accountability. Through the promotion of these principles, we hope that the neuroscience community as a whole can continue to generate high-quality science and build leaders who feel seen, valued and accepted.

With love and respect,

Black In Neuro co-founders Kaela, Rackeb, Elena and Angeline

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RELATED LINKS

Black In Neuro: https://www.blackinneuro.com/

#BlackBirdersWeek, #BlackInNeuro: Black scientists, physicians are using hashtags to uplift: https://eu.usatoday.com/story/life/2020/08/04/blackinneuro-blackinchem-can-hashtags-help-black-scientists-build-community-spotlight-excellence/5541431002/

Society for Neuroscience, Report of Neuroscience Departments & Programs Survey (Academic Year 2016–2017): https://www.sfn.org/-/media/SfN/Documents/Survey-Reports/NDP-Final-Report.ashx?la=en

A Year in Review: Are Diversity, Equity, and Inclusion Initiatives Fixing Systemic Barriers?

Kaela S. Singleton, De-Shaine R.K. Murray, **Angeline J. Dukes**, & Lietsel N.S. Richardson.

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Are current diversity, equity, and inclusion initiatives addressing systemic issues? This article highlights the progress thus far and emphasizes the systemic and cultural shifts needed to support and retain historically excluded scientists.

Conversations surrounding diversity, equity, and inclusion (DEI) have been at the forefront within the neuroscience community in recent months. The pervasiveness of anti-Black racism has been a catalyst for many conversations surrounding the culture and climate of science, technology, engineering, and mathematics (STEM) at large. From the global pandemic that has disproportionately affected marginalized people, to the systemic racism and principles within STEM that deter historically excluded trainees from staying in the field, it has been a year of listening and learning, as well as promises for a better environment: one that supports trainees, understands the concept of intersectionality, and aligns academic excellence with DEI principles. Trainee-driven grassroots organizations have led this charge and birthed a discussion on the importance of DEI principles being incorporated within the scientific enterprise. Now, however, it is time for institutions, both federal and university-based, to support and ingrain DEI commitments into funding mechanisms, tenure and promotion, and academic culture—to create actionable change and move beyond acknowledging the existence of DEI issues and shift to addressing these issues within

academia, the neuroscience community, and STEM as a whole. Academia is a beacon of knowledge and those within it should be the leading lights for cultivating diverse teams. With ideas and perspectives provided by diverse scholars, we can better solve problems and advance research. This cannot happen with the prevalence of stagnant, status quo perspectives on diversity. It requires the expansion of the scope of DEI issues beyond just racial and ethnic identities to encompass nationality, religion, socioeconomic status, disability status, sexual orientation, sex, and gender. Thus, it is time to evaluate the progress made since the release of institutional statements, development of action collaboratives, and formation of DEI committees.

Grassroots trainee-driven movements have spearheaded a push to demand change within the scientific enterprise. These organizations have also embodied the mantra commonly passed down from mentor to mentee: "be the change you want to see." From commencement addresses, panels, conferences, publications, and funding opportunities, the work of early career scientists to improve the culture and climate of academia has been multipronged. Historically excluded scholars have been given a platform to use their voices and share their stories. Conferences hosted by Black In Neuro and NeuroMatch have provided opportunities for scholars to share not just their personal experiences but their scholarly work. This trend has continued with other organizations beginning their own conferences and/or seminar series to highlight the scholarship of Black scientists. Publications from various scholars emphasize the importance of DEI work and have provided resources on how to improve our community at large [1-3]. Further, funding opportunities for historically excluded groups have also been on the rise including Black in Cancer's new program to support Black postdoctoral fellows looking for faculty positions

and the Ben Barres Fellowship sponsored by the National Organization of Gay and Lesbian Scientists and Technical Professionals Inc for trans, intersex, and nonbinary graduate students and postdoctoral fellows in STEM (https://gpchemist.acs.org/opportunities/diversity-and-inclusion/ben-barres-fellowship.html). Collectively, these DEI efforts are empowering trainees on multiple levels: giving students a platform to speak about both their lived experiences and their science, financially supporting them for career growth, and providing spaces to empower the next generation. There are also good-faith efforts in addressing DEI at the faculty level. Cluster hires, from institutes like Mount Sinai's Icahn School of Medicine, aim to provide a sense of belonging and monetary support to increase the number of historically excluded faculty members in a given institution. Furthermore, the new policy by the National Institute of Health on increasing diverse participants in studies and earmarked funding for Diversity R01 grants are also steps in the right direction (https://grants.nih.gov/grants/guide/notice-files/NOT-NS-21-049.html). Most recently, Indiana University-Purdue University Indianapolis has taken these efforts a step forward and begun approving policies to consider DEI work within the tenure and promotion process (https://www.insidehighered.com/news/2021/05/14/iupui-creates-path-promotion-and-tenure-based-dei-work). Collectively, these funding and hiring initiatives prioritize not just the principles of DEI but also high-quality science. These mechanisms also shed light on the importance of resources and money as the academic community embarks on fostering the principles of DEI. By providing these resources in a top-down manner, it signifies that the voices of historically excluded scholars are not just heard but valued and essential to creating a productive and collaborative community. These changes in funding mechanisms,

resources, and culture are the stepping stones needed to recruit, retain, and empower minoritized voices within STEM.

Despite these steps forward, many grassroots movements and historically excluded early career scientists have pointed out major difficulties and setbacks with addressing DEI issues within the academic community: (1) paying early career scientists for DEI service; (2) lack of discussion surrounding intersectionality; and (3) training past, current, and future scholars in DEI practices. Often, DEI committees, panels, and conferences are unwilling or unable to pay historically excluded scholars for their perspectives and voices. Whether it be on university-led committees or panels within an academic conference, compensation for trainees' expertise and energy is essential. Just as an honorarium is provided for scientific seminars compensation should be provided for DEI efforts including panels and workshops. Additionally, the current work-from-home model has shed light on ableism, or discrimination against people with disabilities within the scientific community [4]. From a lack of accommodations for disabled scholars to the "return to pre-pandemic life" movement, institutions have failed to learn about accessibility and incorporate it into their DEI initiatives. This should not be surprising as academia and the scientific enterprise were not built for or with disabled people in mind. However, in order to truly promote DEI, it is necessary to embrace intersectionality and support people's whole identities including their disabilities. This includes recognizing individual and collective struggles and forging policies to ensure equitable, inclusive, accessible and safe working environments. These policy changes should be implemented for both early career scientists and senior researchers and emphasize training in the principles of DEI and understanding the consequences of maintaining a stagnant community. Interestingly, workshops conducted by the National

Academy of Science Engineering and Medicine have demonstrated starkly contrasting opinions from early career scientists and those in positions of power in re-evaluating the training received by postdoctoral fellows specifically (https://www.nap.edu/read/26169/chapter/1). When postdoctoral fellows ask for training on personnel management and/or creating research environments enriched and rooted in the principles of DEI, the response is usually dismissive. There is this myth that all, or at least most, of the issues faced by early career scientists could be solved by picking the "right" mentor or simply extricating oneself from a bad environment. This approach ignores systemic issues and power dynamics within academia as a whole, thereby forcing trainees to undertake the task of creating a better future for academia while giving up emotional labor, time, and resources that could otherwise be used for their academic work.

As we pass the one-year mark of the pandemic, the high-profile murders of Black people at the hands of police across the globe and the promises of solidarity, listening, and learning made by academic institutions, programs, and departments have not been forgotten. In fact, numerous people, most notably Black women, have asked via social media where the institutional changes that were supposed to be forged by DEI promises are. It is in these moments that people in positions of power (whether that be PIs, department chairs, editors, deans, provosts, and directors of funding agencies) should consider the value of their DEI efforts. These conversations have begun already with criticism of recent NIH initiatives (https://www.statnews.com/2021/06/10/nih-releases-plan-to-confront-structural-racism-critics-say-its-not-enough/). Nuanced discussions of all DEI efforts are essential and involve taking a deeper look at each aspect of DEI as it relates to the neuroscience community but also the wider STEM landscape. DEI without the element of diversity results in a

homogeneous and unchanging environment dominated by what is considered "normal" and "professional" [5]; that is, white, cis-heteronormative men or male-dominated culture. Without equity, DEI efforts and policies rely on the free labor of minoritized students, thus resulting in pay inequities that intersect in multiple forms of social identity as well as the inevitable hiring gaps and unpaid labor. Without inclusion, DEI efforts promote tokenism and ostracize the very perspectives it hopes to attract. Success in these three domains also depends on representation and accountability, an effort that many early career scientists are focused on. Without representation in DEI efforts, intersectionality is ignored and results in a loss of diverse voices and perspectives, a lack of policies that address issues facing minoritized early career scientists, and an environment without role models for them. This is best summed up in a quote by Marian Wright Edelman, founder and former president of the Children's Defense Fund: "You can't be what you can't see." The repercussions of a lack of representation and intersectionality can most often be seen when white women are the sole source of diversity in a given environment. Lastly, and perhaps most integral, is the principle of accountability. Without accountability, the scientific enterprise will remain rooted in capitalism and white supremacy, which work together to emphasize a publish-or-perish, profit-over-people, "pull yourself up by your bootstraps"-style of toxic mentorship and career advancement.

In the summer of 2020, scientists from historically excluded groups asked the scientific community to acknowledge the extra work, emotional labor, and effort it takes to exist as minoritized scholars at all levels and take meaningful steps to fix it. The answer to addressing these issues is systemic change within the neuroscience community, scientific enterprise, and STEM as a whole. While progress has been made, there is still a lack of policy

and support that addresses the real issue—the culture of academia. From the lack of consequences for racism, sexism, ableism, homophobia, and transphobia to the deficits in funding, tenure, promotion, and citations, there must be a shift at all levels in academic culture from early career scientists to administrative leadership. This systemic change should start by reimagining a future for academia rooted in the principles of DEI. It requires rethinking the promotion and tenure processes so that they reflect the importance of excellent mentorship, a history of celebrating DEI, and high-quality science. An impressive and informative outline of these steps was recently published for the geosciences [5]. These changes include, at bare minimum, evaluating the current climate; placing Black, Brown, Indigenous, disabled, trans, and/or nonbinary people in positions of power; and giving them the resources and financial support to change policies. Shifting academic culture is also dependent upon providing scientists at all levels with the basic necessities to have fulfilling careers in both STEM and their personal lives. This includes access to proper healthcare, affordable childcare, and parental leave policies, along with salaries and retirement benefits that reflect a livable wage. Making these changes at the graduate level and engaging with those scholars is also critical, since early career scientists often bear the brunt of toxic academic environments [6]. As trainees do not possess the power to force change on their own and are often silenced or ignored when they do speak out against instances of harassment or inequality, it is no wonder that high attrition of historically excluded groups occurs at this stage [7]. From personal experiences and published work, it is clear that minoritized scholars often choose to leave not just STEM but academia as a whole due to the mistreatment and abuse they experience during graduate school [8]. In order to rectify these injustices and retain early career scientists, an overhaul of the toxicity and inequity as well

as academic culture they are exposed to is critical [9]. Without this framework, DEI will continue to be performative in the eyes of historically excluded scientists and they will continue to leave for careers with better compensation and resources.

The systemic changes described above embrace a shift in culture within the field of neuroscience, the academic community, and STEM in order to work toward a solution where scholars of all identities thrive. We must continue to ask this fundamental question: are current DEI initiatives addressing systemic issues faced by historically excluded scientists? Without a systemic shift and culture change where people and their identities are valued more than data, where the product is the person, and the growth they do throughout their scientific career is appreciated, recognized, and rewarded, the answer will continue to be no. Importantly, the goal is to align the principles of DEI with scientific excellence and rigor, not replace them. In fact, studies have shown how the productivity, success, and innovation of research is uplifted when DEI is celebrated [10]. By expanding DEI efforts to include representation and accountability, from both top-down and bottom-up movements, the neuroscience community can change our culture, redefine our values, and ensure that the field represents and celebrates the rich differences within personal identities, benefitting everyone that inhabits our institutions.

Inspiration for this systemic and systematic shift can and should come from the trainee-driven grassroots organizations that are focusing on enriching the lives and scholarship of trainees by going beyond "being the change we want to see" and establishing programs and local communities and creating uplifting content that supports minoritized early career scientists [1]. Organizations such as Black in Neuro, Queer In Neuro, and the Neuroscience Scholars Program are all working to ensure that historically excluded

scientists are retained in order to ultimately enrich academia by embracing and expanding DEI efforts. Thus, those at the top must join in this endeavor by making DEI, representation, and accountability a priority structurally as well as an individual requirement for every academic and begin to carry some of the burden grassroots organizations are currently lifting. DEI changes and policies will never move beyond being performative within the neuroscience community or STEM at large if minoritized early career scientists are continually left to fix the systems of the oppressor. In addition to continuing to listen and learn, action is needed.

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Chapter 5: Summary and Conclusions

NICOTINE USE

The prevalence of nicotine use disorders has become a greater concern as youth drastically increased their use of e-cigarettes and nicotine vape pens in recent years [1]. The younger the exposure, the increased likelihood of developing nicotine dependence, even after occasional intermittent use [2, 3]. Furthermore, this adolescent nicotine exposure could have lasting consequences for adulthood drug and relapse-related behaviors.

Surprisingly, our results indicate that nicotine exposure alone in adolescent mice, regardless of route of administration (injections or vapor), does not alter body weight, anxiety- or depression-associated behaviors, operant learning, or nicotine self-administration during adulthood for either sex. These results are interesting because other studies in the field have found some differences. A study in male rats has shown that adolescent nicotine exposure increases depression-associated behaviors during adulthood [4]. However, these behavioral differences were only found at twice the nicotine doses that we administered. Other studies have also shown that high dose nicotine exposure in adolescent rodents results in reduced weight gain and decreased food consumption in anxiety-inducing conditions [5, 6]. Additionally, regarding nicotine intake, two studies found that rats self-administer nicotine in higher quantities during adolescence which is followed by continued increased nicotine intake throughout the transition to adulthood for females and declining nicotine intake for males as they approach adulthood [7, 8]. Differences in those results as compared to our findings could be due to those studies assessing the progression of nicotine self-administration over time; whereas our studies assessed adolescent exposure followed by a period of no drug exposure before being allowed to self-administer in adulthood. Each of these methods have relevance to human drug use as teens

may continue to use nicotine consistently from adolescence into adulthood or they may quit using nicotine for some time following adolescence and start using again later as adults. Regardless, the period of abstinence before self-administration could have implications on the lasting effects of the adolescent nicotine exposure. Furthermore, as mentioned these other studies generally use much higher nicotine doses than we did. But most human youth who smoke consume one or less than one cigarette per day [9], which is similar to the relatively lower dose that we administered. This lower nicotine dose is also known to be within the rewarding range of the dose response curve and elicit behavioral responses in adolescent mice [10, 11]. Thus, although significant differences were not found among nicotine-only exposed groups in most of our studies, the results are highly relevant to youth experimental patterns of drug use. It is important to note that this does not mean that these low levels of nicotine exposure are harmless in humans. Rather these studies just expand our understanding of the effects of nicotine exposure for these specific measures.

Regarding relapse-related behaviors, the results from our research do indicate that nicotine exposure in adolescence alters cue-induced nicotine seeking in adulthood. This effect is dependent on route of administration in either sex. Specifically, male mice that were exposed to nicotine via injections during adolescence do not exhibit an increase in nicotine seeking across abstinence in adulthood. This is evidenced by similar levels of active lever pressing on day 1 and day 24 of the abstinence period. Adult females that were exposed to nicotine either via injections or vapor during adolescence also do not exhibit this increased nicotine seeking behavior following the 24-day abstinence. In control mice, however, we do see this expected behavioral phenomenon in which nicotine craving incubates over time following withdrawal and re-exposure to an environment with the same drug-associated

cues results in increased nicotine seeking behavior. This lack of incubation of nicotine craving suggests that chronic adolescent nicotine exposure reduces the susceptibility to cue-induced relapse in adult smokers. This interpretation could inform therapeutic interventions for people who did smoke cigarettes or nicotine vape pens in adolescence and are trying to quit smoking in adulthood. Perhaps, drug-associated cues may not be as major of a concern for triggering relapse in this group as other reasons such as stress or users wanting to alleviate the negative withdrawal symptoms.

CANNABINOID USE

The majority of the significant research findings in this dissertation were in relation to the adolescent cannabinoid exposure conditions. Whether exposed to the full cannabinoid receptor agonist, WIN 55,212-2 (WIN), or the partial cannabinoid receptor agonist, Δ^9-tetrahydrocannabinol (THC), during adolescence sex-specific effects were found in adulthood. The findings from this research make it evident that adolescent THC and synthetic cannabinoid use has lasting effects on cognitive-, reward-, and relapse-related behaviors.

Adolescent exposure to a moderate dose of WIN resulted in increased cognitive flexibility in a learning reversal task, decreased anxiety-associated behaviors, and increased natural reward consumption in adult males. However, in females no differences were found regarding cognitive flexibility or anxiety-associated behaviors; but there was a decrease in body weight during the adolescent exposure period as well as a decrease in natural reward consumption during adulthood for those exposed to WIN. The effects of WIN on body weight were transitory, as the difference in females did not persist into adulthood. But the sex-

specific effect on reward consumption is further observed as adult males who were exposed in adolescence to a moderate dose of WIN exhibited increased nicotine self-administration at the lower rewarding nicotine dose in adulthood, but adult females adolescently exposed to WIN demonstrated an opposing effect of decreased nicotine intake.

Prior research findings regarding cannabinoid exposure have variable results. In one study, adolescent exposure to the cannabinoid agonist, CP 55,940, in male and female rats resulted in decreased anxiety-associated behavior when males and females were analyzed together, but these effects were not maintained when males and females were assessed independently [12]. This suggests there may have been baseline differences between the sexes. Interestingly, adolescent exposure to WIN in male Sprague-Dawley rats was shown to increase depressive-like behaviors in the forced swim and sucrose consumption tests [13, 14]. Although, our studies in mice indicate opposing effects, it is important to note that the mice were fully satiated when doing the sucrose consumption test which might explain some of the differences seen in the other studies where the subjects were not. Additionally, in our study, mice were not food restricted during sucrose consumption, in which the opposing differences were found in males and females exposed to adolescent WIN. However, during conditions of food restriction, including operant food training and nicotine self-administration, group differences emerged. Thus, the food satiation levels of the subjects may have implications for the emergence of group differences in these measures.

Across the adolescent drug exposure period, both males and female THC-exposed mice gained less weight, and females exposed to the higher dose of THC had a decrease in body weight. For both sexes, the lower body weight for subjects exposed to the higher dose of THC persisted into adulthood. Females exposed to WIN also decreased in body weight

during adolescent drug exposure, but this lower body weight did not persist into adulthood. These changes in body weight emphasizes the effects of THC on physical development. The results from these studies align with other studies in which chronic THC exposure reduced body weight in rats and prevented weight gain due to reduced energy intake in mice [15, 16]. These findings could be surprising as in humans, THC is often used as a weight gain aid in cancer patients; however, in the general population, THC users tend to have lower body mass indexes [17].

Males exposed to the higher dose of THC earned more food pellets and had more active lever presses during food training and females in this group also had significantly more lever presses during this task. Males exposed to either the lower or higher dose of THC self-administered more of the moderate 0.1 mg/kg/infusion nicotine dose and females exposed to the higher dose of THC self-administered more 0.1 mg/kg/infusion nicotine. These findings are different compared to our previous WIN studies where we did not see any differences in the average food pellets earned in either sex and found increased nicotine intake at the 0.03 mg/kg/infusion dose in males but decreased nicotine intake at both the 0.03 and the 0.1 mg/kg/infusion doses in females. Given that the method of drug exposure, oral as compared to previously injected, and the drug itself, THC compared to WIN, are changed, these differences are not too surprising. Rather, it further adds to the complexity of this story when parsing out the effects of adolescent cannabinoid exposure on later drug-taking behaviors.

Finally, in assessing the impact of adolescent cannabinoid exposure on cue-induced nicotine seeking, we found that both male and female mice that were exposed to the lower dose of THC during adolescence did exhibit enhanced nicotine-seeking as adults following

an extended abstinence period. Furthermore, that males exposed to the higher dose of THC also maintained the incubation of nicotine craving effect which was surprising given the differences in nicotine intake among the male THC exposure groups. However, another study found similar results in which adolescent THC exposure in male mice does not alter adulthood cue-induced reinstatement of nicotine-seeking following extinction [18]. For the female higher dose THC group, while they did not have an increase in nicotine-seeking on day 24 as compared to day 1 of the incubation, they did exhibit an increase in active lever presses on day 1 as compared to their lever pressing during nicotine self-administration. This finding suggests that the higher dose of THC during adolescence may have led to overall increased active lever pressing, which is consistent with the higher level of responding for food training, or it may be a premature incubation effect with higher immediate and persistent drug seeking behavior.

CO-USE CONSEQUENCES

Nicotine and cannabinoid co-consumption is a common occurrence among both tobacco and cannabis users [19, 20]. This co-use condition is of particular importance as co-users have an increased risk of developing both cannabis use disorder and nicotine use disorder [19, 21]. Thus, in all of the studies outlined in this dissertation co-exposure conditions of both nicotine and a cannabinoid during adolescence were assessed.

Exposure to nicotine and a moderate dose of WIN together during adolescence in males resulted in similar effects as WIN exposure alone; namely, increased cognitive flexibility, decreased anxiety-associated behaviors, increased natural reward consumption, and increased intake of a low nicotine dose in adulthood. This suggests nicotine co-exposure

did not produce an additive effect as compared to WIN alone for these behaviors. Conversely, in females, no differences were found for the nicotine and WIN co-exposed mice as compared to control subjects. Because co-exposed females did not have significantly different changes in body weight across the adolescent exposure period, but those exposed to WIN alone did, this suggests that nicotine has some interactive effect in the co-exposure condition that ameliorates this weight loss. This effect can also be found in the sucrose consumption test as exposure to WIN alone reduces natural reward consumption but co-exposure to nicotine restores it to levels similar to control. Interestingly, for nicotine self-administration, females co-exposed to nicotine and WIN earned similar amounts of nicotine infusions as those exposed to WIN alone. The levels of nicotine intake for these groups, however, were significantly lower than females exposed to nicotine alone during adolescence. Thus, in this case, nicotine co-exposure was not able to ameliorate the lasting effects of the synthetic cannabinoid in reducing adulthood nicotine intake.

Previous research studies indicate that in adult mice, chronic co-exposure to both nicotine and THC decreased anxiety-like behaviors at low doses [22]. Similarly, nicotine treatment has been shown to reduce some of the anxiogenic effects of acute THC exposure, and THC treatment can attenuate the anxiogenic effects of acute nicotine exposure [23, 24]. Although we did not assess anxiety-associated behaviors for nicotine and THC co-exposure in our studies, these findings are similar to those found in the WIN experiments for males. For our THC studies, nicotine co-exposure only ameliorated the weight loss effects during adolescence in both males and females co-exposed to the lower dose of THC and nicotine, but not the higher dose. This suggests that the rescuing effects of nicotine are only possible at certain doses of THC. This is further demonstrated in males as the number of food training

rewards earned is not altered by neither nicotine exposure alone nor co-exposure of nicotine and a lower dose of THC, but co-exposure of nicotine and a higher dose of THC does result in more food pellets earned. Interestingly, for nicotine self-administration, nicotine co-exposure with THC at different doses seems to have opposing effects in males. Adolescent exposure to THC at either dose or co-exposure to nicotine and the lower dose of THC increases adulthood nicotine intake, but adolescent co-exposure to nicotine and the higher dose of THC does not. Furthermore, in females, adolescent exposure to a higher dose of THC significantly increases nicotine intake in adulthood. Yet, co-exposure to both nicotine and the higher dose of THC in adolescence does not have any significant effects on nicotine intake in adult females.

Based on the dose of THC, our findings indicate that co-exposure also impacts the metabolism of nicotine. In both males and females, those co-exposed to the higher dose of oral THC and nicotine vapor together had lower levels of cotinine, a metabolite of nicotine, in their blood following the adolescent drug exposure paradigm than those exposed to nicotine vapor alone and those co-exposed to the lower dose of THC and nicotine vapor. This finding indicates that the higher dose of THC may impact the metabolism of nicotine. In support of these findings, another study confirms that in human smokers, co-users of both nicotine and THC have lower cotinine levels than tobacco only smokers [25]. In regards to the impact this co-use condition has on relapse-related abstinence, adult co-users are twice as likely as tobacco smokers who do not use cannabis to continue smoking tobacco [26]. This could be due to the cannabinoids enhancing the effects of nicotine-associated cues in reinstating the drug-seeking behavior after a quit attempt [27]. This notion is supported by other studies that demonstrate how administering a cannabinoid receptor antagonist

decreases cue-associated nicotine seeking behaviors [28, 29]. Our incubation of craving findings further supports this theory as adolescent exposure to nicotine vapor or the higher dose of THC in females prevents enhanced nicotine-seeking in adulthood following an extended withdrawal period; but surprisingly, adolescent co-exposure to nicotine vapor and oral THC at either dose does result in this incubation effect. Thus, the interactive effects of nicotine and THC allow this incubation of nicotine craving phenomenon to occur despite single-drug exposure preventing it. For these poly-drug exposure conditions, if cannabinoids enhance the effects of nicotine-associated cues in reinstating drug-seeking behavior, as previously mentioned, then the adolescent co-exposure to both drugs could make the subjects more sensitive to the drug-associated cues later in life and more prone to relapse.

In the future, additional studies will need to be conducted to determine the underlying mechanisms of the incubation of craving findings for both the single and poly-drug exposure conditions. The prefrontal cortex, ventral tegmental area, nucleus accumbens, and amygdala are brain regions of interest for these studies as cannabinoid and nicotinic acetylcholine receptors exhibit overlapping expression within them [30, 31]. But the amygdala in particular is an area that has strong implications for potential mechanisms underlying the incubation of craving effect., c-Fos expression in the amygdala is strongly enhanced by co-administration of nicotine and THC [22]. Human fMRI studies have also revealed an increase in amygdala activity when nicotine-deprived smokers were shown smoking-related images [32]. Furthermore, the basolateral and central amygdala have been shown to be involved in memory reconsolidation of cues previously paired with drug self-administration and nicotine seeking, respectively [33-35]. For these reasons, each of these

brain regions, especially the amygdala, warrant future experiments to parse out the mechanisms underlying this nicotine, cannabinoid, and poly-drug exposure.

CLINICAL IMPLICATIONS FOR TREATING SUBSTANCE USE DISORDERS

When analyzing the results of the studies from this dissertation from a clinical standpoint, there are several implications in regards to the approach and personalized treatment of individuals suffering from nicotine, cannabis, and these co-occurring substance use disorders. Our studies in mice have demonstrated that while nicotine vapor exposure alone in adolescence does not alter nicotine intake in adulthood, co-use with cannabinoids does increase nicotine intake. Adolescent WIN and THC exposure in males as well as higher dose THC exposure in females also increases nicotine intake in adulthood. Therefore, people who consumed cannabinoid-containing products or co-used nicotine and cannabinoid products during adolescence may be consuming more nicotine in adulthood and be more susceptible to developing nicotine use disorders.

Furthermore, our studies in mice have also shown that after consistent nicotine self-administration, pretreatment with a low dose of a cannabinoid can reduce nicotine intake in both males and females. However, in that same study if the mice were exposed to nicotine, cannabinoids, or both during adolescence, this effect would not occur. Thus, prior drug history may be a mediating factor in the effectiveness of pharmacological cessation treatments. Other preclinical and clinical studies have also looked into cannabinoids as a potential smoking cessation treatment. Interestingly, antagonists of the cannabinoid receptor, as opposed to the agonist WIN that we used in our studies, have also been shown in rodent models to decrease nicotine self-administration and reduce nicotine-induced

dopamine release in the nucleus accumbens [36]. In clinical trials, two different cannabinoid antagonists, rimonabant and taranabant, were found to be marginally effective for smoking cessation; but they had to be withdrawn from the market due to increasing anxiety and depression in the participants [37, 38]. The notable occurrence of which both cannabinoid receptor agonists and antagonists could be effectively used for nicotine cessation treatments can be explained by the retrograde feedback mechanism of action that cannabinoid receptors function. Since cannabinoid receptor binding suppresses the release of neurotransmitters, agonist binding could result in that dampening effect if the receptors are on glutamatergic neurons [39]. But also, antagonists binding could prevent the binding of endogenous cannabinoids which could result in a dampening effect if the cannabinoid receptors are on a GABAergic neuron, such as those in the ventral tegmental area [40].

Building upon the importance of considering prior drug history in treating substance use disorders, those who used either of these drugs in adolescence may have an altered responsivity to drug-associated cues in adulthood. Specifically, our studies have shown that those who use either nicotine or THC could be less responsive to cues that are associated with later nicotine-taking and therefore less susceptible to cue-induced relapse. These findings are important for personalized approaches to treating substance use disorders for people who have a history of using either drug in adolescence and are now trying to quit nicotine use in adulthood. These patients' treatment programs may need to focus on other triggers of relapse besides cues. It is also important to emphasize that although this lack of responsivity for drug-associated cues to trigger relapse can be seen as a positive effect of adolescent nicotine or THC use, there are still many negative consequences to adolescent drug exposure including increased anxiety, depression, and intake of other drugs following

nicotine exposure as well as cognitive deficits in attention, learning, and memory following cannabis use [41, 42]. Additionally, this finding does not mean that teens who use nicotine will not relapse after quitting smoking in adulthood. Rather, these findings specifically indicate that they may be less susceptible to cue-induced relapse but other factors such as stress or negative withdrawal symptoms may still trigger relapse.

For the treatment of cannabis use disorder, studies indicate that synthetic cannabinoids or therapeutics focused on nicotinic receptors may be beneficial. In rodent models, administering an antagonist of nicotinic receptors reduces self-administration of a synthetic cannabinoid and prevents THC from increasing dopamine in the nucleus accumbens shell [43]. This provides evidence of the reciprocal impacts cannabinoid and nicotine receptors have on each other and suggests that nicotine receptor antagonists could be a potential therapeutic for treating people with cannabis use disorders. Low-dose nicotine patches have also been shown to reduce negative affective cannabis withdrawal symptoms but only in subjects that were not heavy tobacco users [44]. As evidenced by this study, the co-use of both substances presents unique challenges in the cessation of either one. Daily cannabis users who also smoke tobacco cigarettes have higher rates of cannabis relapse [45]. Similarly, cannabis use decreases the likelihood of tobacco cessation [26]. Reducing the use of both substances may be beneficial in the cessation of the other. This is evidenced by a study that found people attempting to quit or reduce cannabis intake also reported using less tobacco on abstinent days [46]. Thus, research on effective cessation methods for co-users will aid in the smoking cessation of people suffering from nicotine, cannabis, or these co-occurring use disorders.

Additionally, assessment of trends in human subjects reveal that co-occurring nicotine and cannabis use disorders are linked with an increased risk of other mood disorders and psychiatric diagnoses. Adults with both nicotine and cannabis use disorders are more likely to have bipolar disorders, anxiety disorders, and personality disorders [47]. Among youth and young adults, nicotine and cannabis co-use increases the risk of psychotic experiences [48, 49]. They are also seven times more likely to have any psychiatric diagnosis than non-users [50]. Consequently, understanding the similar neurobiological mechanisms common to these psychiatric conditions and substance use disorders may identify unique targets for future therapeutic developments.

CONSIDERATIONS FOR COMMUNITY IMPACT

As we conduct addiction-related research on the lasting effects of nicotine and cannabis use, it is important to bear in mind the people that this research will impact. There are systemic inequalities and blatant racism that is associated with the stigma, incarceration, and treatment of people with substance use disorders. Therefore, there are major implications of how drug addiction research affects historically marginalized communities.

Cigarettes, in particular mentholated cigarettes, have always had major racist underpinnings. In the early 1900s, tobacco companies attempted to increase cigarette consumption by offering a wide variety of flavoring options, like cherry and chocolate, which were appealing to young people. To dissuade them from initiating smoking, the U.S. Food and Drug Administration banned all flavored cigarettes, except for menthol, in 2009. This was effective in reducing youth and young adult cigarette use over time [51]. Concerningly, as mentioned menthol cigarettes were not initially banned. The cooling sensation provided

by mentholated cigarettes make them easier to smoke and harder to quit. They were primarily used by vulnerable populations including people in the Black community, those with a lower socioeconomic status, and those suffering from mental illness [52]. Specifically, tobacco companies targeted marketing of these cigarettes to predominantly Black neighborhoods. This resulted in immense health disparities with Black people being more likely to die from smoking-related diseases even though they smoke fewer cigarettes than their White counterparts [53]. In 2021, due to mounting pressure related to social justice movements and neuroscience research findings that mentholated cigarettes have implications in nicotine addiction, the FDA finally banned menthol flavoring in cigarettes [54-56]. However, the inequalities associated with the ban is still heavily debated because although the ban is not enforced against consumer possession of these cigarettes, it increases the risk of police violence and criminalization of people on the street accused of selling them as a continued consequence of the "War on Drugs".

The "War on Drugs" began in the 1970s to reduce illegal drug use through increased policing and mandatory prison sentencing. Since its conception, there has been consistent disproportionate harm to and arrests of people from Black, Hispanic, and impoverished communities for drug-related offenses. Over 70% of people in prison for drug-related offenses are Black or Hispanic [57]. This is not to say that these populations use drugs more. Rather, despite having similar rates of drug use and distribution as their white counterparts, they are more likely to be searched, arrested, and receive harsher sentencing for the same offenses [58, 59]. Cannabis especially has been a scapegoat as a reason for the incarceration and criminalization of people from marginalized communities with the vast majority of drug arrests from 1990-2002 being low-level cannabis possession charges [60]. Even in recent

years, Black people are still disproportionately arrested 3.6 times more often than whites for cannabis-related offenses across the United States [61]. While these might seem like merely criminal justice issues and unrelated to neuroscience, the fact is that the research we conduct in drug abuse influences the way the general community, the government, and the justice system views addiction.

The focus scientists tend to have on emphasizing the detrimental effects of substance use to acquire grant funding has very serious consequences on the people battling these diseases and the way law enforcement perceives them. As such, if someone is suffering from a substance use disorder, they may be more readily dismissed as untrustworthy, deemed as a criminal, or viewed as an unfit parent. Dehumanizing drug users is problematic to say the least and ruins countless lives. We must be cautious in our language as to not overinflate the harms from our findings and be honest in the potential positive or neutral outcomes as well. It is imperative that we as scientists bear in mind the impact our research has on the people and policies it relates to and strive to not cause further harm to vulnerable communities through our work.

VALUING DEI IN AND OUT OF THE LABORATORY

Lastly but certainly not least, it is important to bear in mind who is conducting this research and who is represented in the studies. As the findings from addiction research have disproportionate impact in Black and Brown communities, both the patient populations being assessed and the researchers themselves should be reflected. In the participants with whom the research is being conducted, it is crucial that they are representative of the larger population in order to draw accurate conclusions. This presents a unique challenge as there

is a long-standing history of reasonable mistrust that people from historically marginalized groups have when it comes to research [62-64]. Thus, having research groups with a collective of diverse scientists helps promote trust among participants as they are more readily able to connect with people from their same racial/ethnic backgrounds [65]. This is also important for the dissemination of research findings as persons belonging to these groups are better able to present the results to the broader community in a culturally inclusive manner. Beyond just race, it is beneficial to both the research and the research environment to have researchers and participants from a breadth of age ranges, gender identities, socioeconomic statuses, nationalities, and cultures. Welcoming these diverse groups strengthens research by bringing in a wealth of unique perspectives that can improve the ways we approach scientific problems.

As we discuss diversity, equity, and inclusion (DEI), however, we must note that it goes further than just recruiting diverse persons to be involved in research. Diversity ensures everyone is properly represented. But equity requires removing the systemic barriers that prevent certain groups from being able to actively participate. And inclusion is ensuring that everyone has a voice, and that their perspectives are heard and valued. Each of these principles are imperative for the continued progress of scientific research and the improvement of academia at large. Cultivating more equitable, diverse, and inclusive laboratory environments requires providing opportunities for historically marginalized persons to participate and share their insights. This involves providing access to funded research opportunities, taking into consideration other factors that might impact research productivity when evaluating candidates for graduate school admissions or other career opportunities, and creating non-discriminatory, safe research environments that provide the

resources necessary for growth. It also involves actively removing systemic barriers that prevent this progress from occurring. Moreover, it requires valuing the community service, outreach, and mentoring work that historically marginalized scholars do to build trust with the community, ensure the retention of trainees, and integrate the next generation of historically-excluded scientists. Black and Brown scholars deserve to be supported and valued for both their research and their contributions to making science more accessible. To do so, they need the proper mentorship and structures in place that demonstrate it is valued when it comes to graduation, promotion, and tenure. There needs to be a shift in research and academia that goes beyond just making statements about the importance of DEI, but that makes systemic changes to actually allow it to thrive. In doing so, our research, our laboratories, and our communities will be all the better.

CONCLUSIONS

In sum, the findings from the studies outlined in this dissertation provide an overview of the lasting effects of adolescent nicotine and cannabinoid use. Our findings demonstrate that adolescent exposure to a synthetic cannabinoid or co-exposure to both nicotine and a synthetic cannabinoid alters anxiety-related behaviors, cognitive flexibility, natural reward consumption, and nicotine intake in a sex-dependent manner. Additionally, that exposure to the cannabinoid THC or co-exposure to nicotine and THC alters food and nicotine intake dependent on dose and sex. And finally, our studies demonstrate that adolescent drug exposure can impede the effectiveness of potential smoking cessation therapeutics and alter the responsivity to cue-induced drug seeking later in life.

This research has significant impact on the field of addiction neuroscience by revealing some of the lasting consequences of adolescent nicotine and cannabis use on cognition, later drug intake, and relapse-related behaviors. Beyond single drug use, this research also provides valuable insights into the subsequent unique effects occurring from co-exposure to both of these substances of abuse. Moreover, this work can further inform the treatment of patients seeking care for single or co-occurring nicotine and cannabis substance use disorders. The novel findings from these dissertation studies give insight into how patients may differentially respond to pharmacotherapeutics based on adolescent drug exposure and inform a potential underlying factor mitigating individual differences in cessation outcomes.

Finally, this dissertation highlights importance of having diverse perspectives in the field of neuroscience, how to better support historically marginalized scholars, and the necessity of being cognizant of how our research impacts the community.

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