Psychosocial and Biochemical Correlates of Nicotine Administration via Vaping Behavior

A Major Qualifying Project Report
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In partial fulfillment of the requirements for the
Degree of Bachelor of Science by:

Mira Kirschner

Advised By:

Professor Angela C. Incollingo Rodriguez, Ph.D.
Department of Social Science and Policy Studies

Professor Jagan Srinivasan, Ph.D.
Department of Biology & Biotechnology, Neuroscience Program

ABSTRACT

E-cigarette use is widely prevalent among young adults. However, there is not much known about the social consequences of nicotine in this age-group. This two-part interdisciplinary project (a) delivered a psychosocial survey focused on vaping behaviors and mental health that was administered to 173 WPI undergraduates and (b). measured social behavioral responses to nicotine exposure in a model organism, *C. elegans*. Key findings across both studies include the following: 35% of respondents reported they vaped with some degree of regularity. Vaping appears to be a social activity among WPI students and less-so related to psychological mood states. However, younger vapers tended to have higher depressive symptoms. *C. elegans* exhibit avoidance behavior to increasing concentrations of nicotine. This project emphasized that although vaping is a social activity, there are behavioral consequences at the biological level.

ACKNOWLEDGEMENTS

I would like to thank both of my advisors, Dr. R and Dr. J, for being my cheerleaders throughout this solo project. Without them, I would not feel proud about my project. They helped me feel confident in my work, reminded me to look on the bright side, and were very understanding.

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I also want to thank the graduate students in Dr. J's lab, specifically Caroline Muirhead, for teaching me worm maintenance, how to perform avoidance assays, and sparing me extra worms when mine either got contaminated or died after I got sick.

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INTRODUCTION

The use of e-cigarettes in the United States has been on the rise since being introduced to the market in 2007. E-cigarette use among youths increased 900% during 2011 to 2015 (CDC, 2018). E-cigarettes have been used as a cigarette smoking cessation tool but differ from cigarettes in keyways: E-cigarettes use juice with varying concentrations of pure nicotine, whereas cigarettes use nicotine mixed with tobacco. Public perception has determined that vaping is less harmful and poses no significant threat (Dinardo & Rome, 2019). However, there is limited research evidence to confirm this public perception, which is concerning given the high prevalence of use. It is important for research to investigate the psychological, social, and biological consequences of vaping.

The present project sought to fill this gap through investigating both the psychosocial predictors and correlates of vaping in humans and the behavioral consequences of nicotine use in a model organism, *C. elegans*. More specifically, this interdisciplinary project focused on (a) surveying college students (a population that exhibits high rates of vaping behavior (Karamarow & Elgaddal, 2023)) to determine what moods and social factors surround vaping behaviors and (b) experimentation with *C. elegans* to determine how varying concentrations of nicotine may affect social behaviors at a biological level through the avoidance response. This research aimed to provide beneficial insight into the predictors, correlates, and consequences of vaping in college-aged students.

BACKGROUND

The following sections provide an overview of the consequences of vaping on the body and mental health, how nicotine affects receptors in the brain; and how *C. elegans* have been used as model organisms to look at nicotine-related behaviors.

2.1 Biological and Downstream Health Consequences of Vaping

Typically, the terms “vapes” and “e-cigarettes” are used interchangeably, but they are also two different types of devices. As seen below in Figure 1, vapes are refillable, can be recharged, and the flavor can be switched out. E-cigarettes are disposable and come with a predetermined flavor. With both vapes and e-cigarettes, the user inhales vapor rather than smoke, hence the term “vaping”. As of 2022, one in four adolescents use e-cigarettes daily. A majority (84.9%) use flavored e-cigarettes, with the flavor breakdown as follows: fruit (69.1%), sweet (38.3%), mint (29.4%), and menthol (26.6%). Looking at adults, about 4.5% of them use e-cigarettes. Adults in the 18-22 age range have the highest level of vaping at 11% (Kramarow & Elgaddal, 2023). As of 2016, more adults and adolescents have reported using e-cigarettes over smoking actual cigarettes (Dinardo & Rome, 2019). As e-cigarettes rise in popularity, there is still more research that needs to be done into how mental health plays a role.

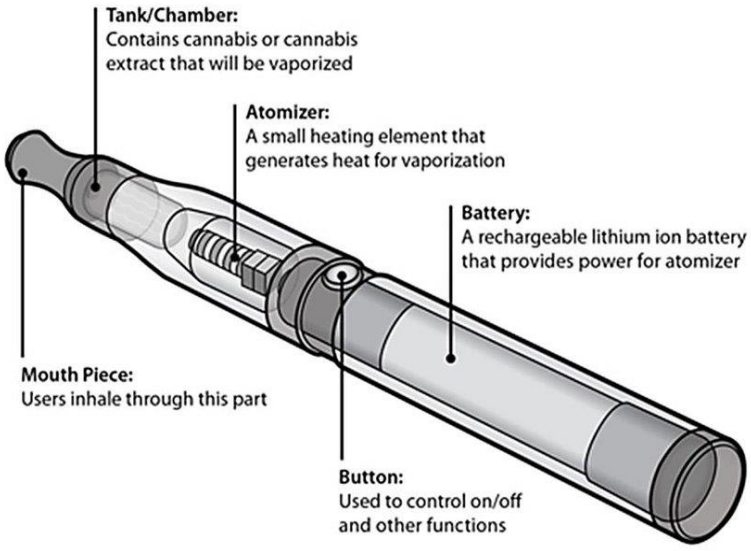

Figure 1. *Typical vaping device components (Bility, 2019)*

A study conducted in the United States examined 32,636 adolescents across grades 8, 10, and 12 and looked at potential links between e-cigarette usage and depressive symptoms (Gorfinkel et al., 2022). As the grade level increased, researchers found that the prevalence of vaping had also increased. They found that 13.1% of the adolescents with depressive symptoms (N= 28,546) vaped nicotine but did not use cigarettes, whereas 4.1% vaped nicotine and smoked cigarettes, and only 2.4% smoked just cigarettes. This suggests that adolescents with depression may be more prone to vaping rather than using cigarettes.

As e-cigarettes continue to rise in popularity, the devices evolve. New generation vaping devices can heat the liquid to a higher temperature, which, in turn, releases more nicotine, making it a more efficient form of nicotine administration (Dinardo & Rome, 2019). Nicotine is the main substance of vaping liquid and can increase the risk of cardiovascular, respiratory, and gastrointestinal diseases (Mishra et al., 2015).

However, nicotine is not the only potentially active ingredient implicated in vaping. The vaping liquid, colloquially termed “vape juice,” contains various other toxic substances, such as

reactive aldehydes, acetone, and carcinogenic nitrosamines. High levels of reactive aldehydes from both cigarettes and e-cigarettes are to blame for 33% of deaths due to cardiovascular disease (Dinardo & Rome, 2019). Additionally, reactive aldehydes are linked to a higher risk for the onset of lung, oral, and gastrointestinal cancer (Sinharoy et al., 2019).

It has also been found that fruit flavored e-cigarettes cause a significant increase in DNA fragmentation, which is when DNA strands break into pieces (Dinardo & Rome, 2019). This is essentially programmed cell death, or apoptosis. Higher levels of DNA fragmentation have been correlated with male infertility and higher miscarriage rates (Simon et al., 2019).

Along with DNA fragmentation, e-cigarettes provoke alterations of DNA repair systems through oxidative stress (Tobore 2019). Oxidative stress occurs when there is an imbalance of oxygen reactive species resulting in the body's inability to detoxify and repair any damage. These oxygen reactive species are key cell signaling agents and play a significant role in substance addiction/dependence. E-cigarette induced oxidative stress is connected to various harmful effects such as depression, social-behavioral deficits, and suicide ideation (Tobore, 2019). Tobore (2019) also found that e-cigarette usage also increases psychological distress, which has been connected to increased odds of depression.

2.2 Psychosocial Predictors and Correlates of Vaping

Due to the rising prevalence of vaping and the negative consequences, increased attention is being paid to the psychosocial correlates of vaping. One study looked at highschoolers in Massachusetts. Dube et al. (2023) sampled eleven students from a suburban high school who vaped in the past 90 days and conducted semi-structured interviews. They found that peers had some sort of influence, as vaping initiation tended to occur when around friends who also vape. New flavors seemed to encourage vaping behavior and the fact that it was "something to do" as

an idle activity. Vaping was coupled with playing games, getting ready to go out, talking on the phone, and studying. The main reason these high schoolers continued to vape was to help control anxiety symptoms.

Though research has investigated which age groups are more likely to vape, there is little known about the psychosocial predictors and correlates of vaping behavior in college-aged adults. For example, under what circumstances are they more likely to start vaping? What mood states are associated with these vaping behaviors? What leads these prominent age groups to begin vaping? Studies have shown that depression plays a factor in vaping (Oliver et al., 2023), however, not many studies have determined whether loneliness plays a significant role (Dyal & Valente, 2015). There is also not much known about how nicotine drives these social behaviors through biochemical pathways and which pathways may be involved.

2.3 Biochemistry of Nicotine Use and Addiction Across Age Groups

Nicotine is introduced to the body through the lungs, before passing through the blood-brain barrier and biological membranes (Zhang et al., 2022). When comparing nicotine use in adolescents versus adults, research has found that nicotine induces a greater release of dopamine in adolescents due to the immaturity of the receptors (Leslie, 2020). Consistent exposure to nicotine can desensitize these dopamine receptors over time (Balfour & Ridley, 2000). This drug stimulates dopamine-secreting cells that project to the mesocorticolimbic system. This system includes various structures, such as the prefrontal cortex, which plays a role in impulsive behaviors, and the nucleus accumbens, which modulates the feeling of reward. Balfour & Ridley (2000) believe that these projection pathways are believed to play a role in the development of dependence to drugs like nicotine. They found that nicotine can also exert antidepressant-like activity by causing a release of dopamine by acting on nicotinic acetylcholine receptors

(nAChRs). These receptors have been found to play a crucial maturation role in various development stages (Leslie, 2020). However, the neuronal nicotinic receptors that are expressed on the dopamine-secreting neurons become desensitized over time due to consistent exposure. This means that, eventually, a higher concentration of nicotine is necessary to exert the same effect on the dopaminergic receptors. Although nicotine results in a temporary burst of dopamine, it can lead to adverse effects, such as increased heart rate and blood pressure (Gurusamy & Natarajan, 2013). Not only does nicotine affect blood pressure, it affects the bloodstream itself. Nicotine increases the amount of glucose in the blood and the levels of catecholamines, which are hormones released in the body in response to stress (Mishra et al., 2015).

Nicotine addiction leads to upregulation of nAChRs due to an increase in the number of receptors after continuous exposure (Ortells & Barrentes, 2010). Upregulation is a characteristic of α4β2 receptors, which are the most common nAChRs in the brain and have direct involvement in nicotine addiction. High affinity nicotine binding sites in most of the brain regions are formed from this type of nAChRs. Nicotinic acetylcholine receptors have three different states: resting, activated, and desensitized. The receptor channels are closed, and binding sites are empty in the resting state. The channel temporarily opens upon binding of a ligand of high-agonist concentrations. After the activated state, it is less responsive or considered desensitized due to the binding sites being occupied at a high affinity. Once the agonist is removed, the receptor goes back to its resting state. Low agonist concentrations have been shown to trigger a process called "high affinity desensitization" that affects α4β2 receptors in the presence of nicotine where desensitization is induced without activation. Desensitization is also thought to trigger

upregulation, as it is a homeostatic response to desensitization. However, desensitization can also be seen as a determinant of nicotine tolerance.

2.4 Caenorhabditis elegans as a Model Organism for Studying Nicotine

One way to begin learning about how nicotine drives social behaviors, and which biochemical pathways are affected is through the use of a model organism. Caenorhabditis elegans, also known as *C. elegans*, is a commonly used model organism to study neurobiology and molecular pathways. They are easy to culture and easy to work with in the lab. They require minimal nutrition and do not have demanding growth requirements. *C. elegans* also emulate certain aspects of human pathology, as they contain complex molecular pathways and genes that are also found in humans. They also have a short life span, which makes it easy to track and follow development. *C. elegans* have fostered better understanding of various diseases, including depression (Kaletta & Hengartner, 2006). Their genomic information is widely available online, making them an excellent resource for testing effects on various pathways and behaviors.

2.4.1 Dependent Behavior

Past research has exposed *C. elegans* to nicotine and looked at dependent behavior. These nematodes have shown similar behavioral habits when exposed to nicotine as mammals. After being exposed to nicotine for a prolonged period, they developed a tolerance and began to behave similarly to the nicotine-free worms (Feng et al., 2006). When the worms were placed on plates with nicotine, they exhibited an increase in locomotion speed, which has been denoted as “locomotion stimulation”. Their response was dose-dependent, with the peak response being at a 1.5 uM dosage. When the researchers looked at withdrawal symptoms, they found that the worms displayed similar locomotion-stimulation behavior (Feng et al., 2006). However, other

research has stated that the effect of nicotine on *C. elegans* could be due to the nicotine negative affecting the food quality rather than something happening to the nicotinic receptors (Kudelska et al., 2018). The researchers also concluded that the effects of nicotine vary depending on the stage of development the worm is at, as well as the dosage. Along with a change in locomotion speed, nicotine has been found to cause stimulating egg-laying effects where *C. elegans* exposed to nicotine tended to lay eggs earlier in development. Chronic exposure to nicotine led to an insensitivity to this stimulation effect, suggesting desensitization of the nAChRs (Polli et al., 2015).

2.4.2 Avoidance Response

Avoidance behavior occurs when faced with an external, threatening stimulus. In humans, this can cause activation in the amygdala, which regulates the innate fight-or-flight response. In both humans and nematodes, avoidance behavior can be considered a self-preservation tactic to protect the body from potential harm. Due to *C. elegans* having limited senses, they heavily utilize their nervous system and rely on chemo-sensation to detect and respond to threatening stimuli.

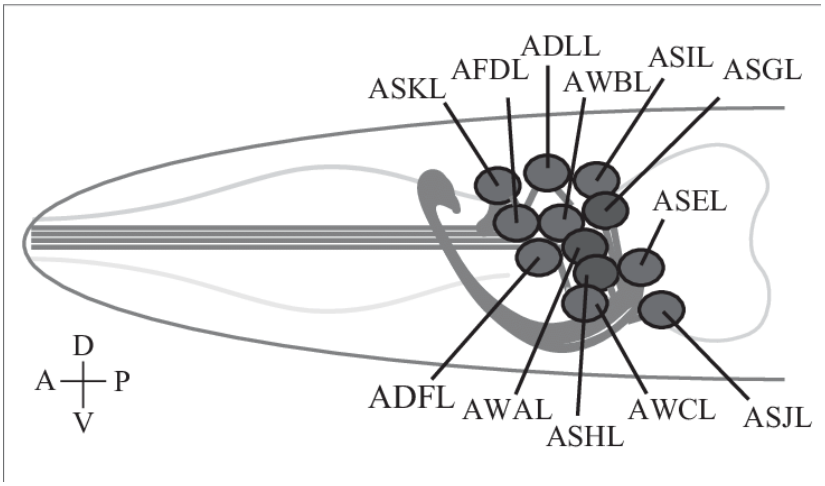

Figure 2. *Sensory neurons in the amphid (head) (Maruyama, 2017)*

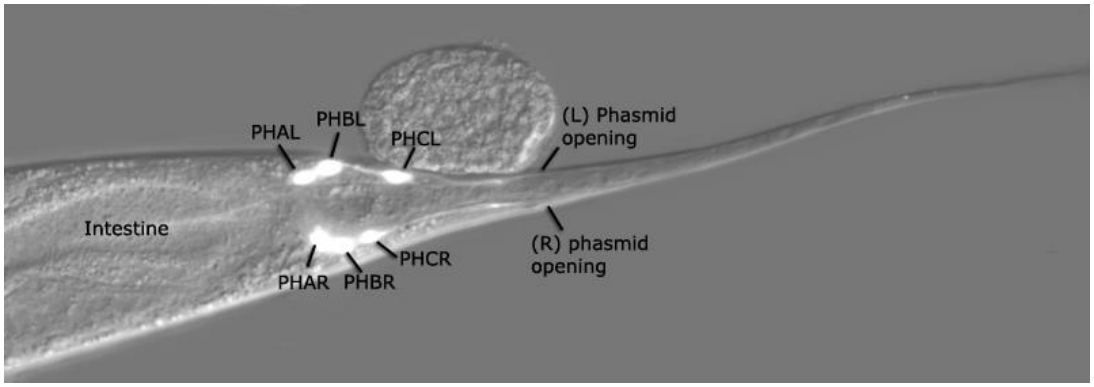

Figure 3. *Sensory neurons in the phasmid (tail) (WormAtlas, n.d.)*

C. elegans have a small nervous system with only 302 neurons. The amphid is the largest sensory organ in the nematode and consists of 12 sensory neurons (Figure 2) capable of detecting various stimuli (Zou et al., 2018). The phasmid is a sensory organ located in the tail of the worm (Figure 3) and contains similar structures to the amphid. Hilliard et al. (2002) found that antagonistic activity in the amphid and phasmid neurons unite to generate avoidance behaviors. The avoidance response in *C. elegans* is characterized as the cessation of forward movement, followed by backward reversals.

C. elegans have been a consistent model organism in both biological and neuroscience studies when observing the effects of nicotine. They have shown behavioral deficits when exposed to nicotine, such as reduced egg-laying behavior and withdrawal symptoms. However, there is little research done on the avoidance response to nicotine as a threatening stimulus.

2.5 Goals and Hypotheses

Multiple pressing questions emerge from the gaps in the above reviewed literature: a) What are the psychosocial predictors of vaping in college-aged students? b) Why do students choose to begin and continue vaping? and c) How does nicotine affect social behavior? To fill these gaps, the present work executed an interdisciplinary arc of comparative studies in both

humans and animal models looking at the social consequences of nicotine administration. The first study examined which psychosocial factors are predictors of vaping behaviors, the overall trends in vaping on the college campus and conditions under which students initiate and continue to vape. The second study investigated whether nicotine would be an aversive stimulus to *C. elegans* by testing avoidance behavior with varying concentrations of nicotine.

METHOD

This work contained two complementary studies: Study 1, a psychosocial survey of vaping use among college students and Study 2, an experimentation with *C. elegans* to measure the biological implications of nicotine use.

3.1 Study 1: Vaping Survey

3.1.2 Participants

Participants were undergraduate students at Worcester Polytechnic Institute (WPI). They were recruited through the WPI Psychology subject pool administered through Sona. Participants were compensated with 0.5 research credits that went towards their final grade in a Psychology course.

3.2.2 Procedure

This study was approved by the university's Institutional Review Board. Each participant completed a survey through Qualtrics, a secure, online survey software (Qualtrics, Provo, UT). Before beginning the survey, participants were instructed to read an informed consent document and agreed to participate by selecting "I agree to participate in this research." Participants who

consented were asked to answer a series of questionnaires (described below). Data was collected from October of 2023 through February of 2024. Once the data collection period ended, responses were moved from Qualtrics to a secure WPI server, where all data management and analyses were performed.

3.2.3 Measures

Demographics. Participants reported their age in years, race/ethnicity (White, Asian, Pacific Islander, Black or African American, American Indian or Alaska Native, Hispanic or Latino/a, Multiple Races, Prefer not to say, Other), gender identity (Male, Female, Transgender, Gender-fluid, Non-binary, Prefer not to say, Other), and their planned graduation year.

Vaping Behaviors. Participants were asked to fill out various questions relating to their vaping behaviors and their friends' vaping behaviors. Participants who reported “yes” when asked whether or not they vape then received follow-up questions: “What concentration do you typically vape (in mg)?” “During which occasions or situations do you typically vape?” “Why do you vape?” and “What led you to begin vaping?” Participants who reported “no” were asked the following questions: “Have you ever felt pressured to start vaping?” and “Why do you choose not to vape?” All participants, regardless of vaping history, were asked about their general opinion of vaping, with responses ranging from “extremely negative” to “extremely positive.” They were also asked whether their friends vape and how many of them vape. Questions were designed based on conversations that occurred either in-person or over the phone with 12 current college students when asked about their vaping tendencies and through consultation across the research team. See Appendix A for a complete list of questions and response options.

UCLA Loneliness. The 20-item UCLA Loneliness Scale (Russell et al., 1978) was used to assess feelings of both loneliness and social isolation. For example, one item stated, “I am unhappy

doing so many things alone." Participants reported their feelings on a 4-point Likert scale, ranging from "never" to "often". A higher total score indicated greater feelings of loneliness and social isolation. Cronbach's alpha in this case was 0.95.

PHQ8. To evaluate potential symptoms of depression over the previous two weeks, participants completed the 8-item Patient Health Questionnaire-8 (PHQ8) (Kroenke et al., 2001). A sample item reads, "Over the last two weeks, how often have you been bothered by little interest or pleasure in doing things?" Participants reported their feelings and behaviors on a 4-point Likert scale ranging from "not at all" to "nearly every day". A higher total score indicated more depressive symptoms. Cronbach's alpha in this case was 0.91.

GAD7. The General Anxiety Disorder-7 (GAD7) questionnaire (Spitzer et al., 2006), measured the severity of anxiety over the last two weeks. Participants reported their feelings and behaviors by responding to questions such as, "Over the last two weeks, how often have you been bothered by feeling nervous, anxious, or on edge?" Items were scored on a 4-point Likert scale, ranging from "not at all" to "nearly every day". Total scores ranging from 16-22 indicated severe anxiety. Cronbach's alpha in this case was 0.90.

Perceived Stress. To measure general feelings of psychological stress, participants completed the 14-item Perceived Stress Scale (Cohen et al., 1983). The questions asked about their stress-related feelings and thoughts during the last month. The scale included both negative and positive items, such as "In the last month, how often have you felt nervous and stressed?" and "In the last month, how often have you felt that things were going your way?" Participants reported their feelings on a 5-point Likert scale, ranging from "never" to "often". A higher total score indicated higher perceived stress. Cronbach's alpha in this case was 0.82.

3.2.4 Analytic Plans

Data from the survey were cleaned and analyzed using IBM SPSS Statistics 29. A total of 173 responses were collected, however, 36 responses were excluded from data analysis due to incomplete responses. Correlation analyses were used to examine the continuous relationships between the following variables: loneliness scores, depression scores, anxiety scores, stress scores, and age. ANOVAs examined differences in mental health measures based on age and whether someone may vape. Regression analyses examined whether mental health measures were continuous predictors of vaping. Microsoft Excel was used to count demographic data, reasons for vaping, reasons why the participant does or does not partake in vaping, typical vaping settings, and favorite vape flavors. T-tests were used to compare the average avoidance index in different percent nicotine groups.

3.3 Study 2: C. elegans Experimentation

3.3.1 Strains and Worm Maintenance

The N2 wild-type strain of *C. elegans* were picked onto 6cm Nematode Growth Medium (NGM) plates. These plates were prepared by pipetting 1-2 mL of OP50 onto the plate and stored at room temperature until dried. The *C. elegans* were transferred onto these plates using a sterile titanium pick. These plates were then stored in a 16°C refrigerator. *C. elegans* were transferred to new seeded plates at least twice a week.

3.3.2 Avoidance Assays

Open NGM plates with worm populations were left to dry in a 16°C room for 20 minutes before the experiment was conducted. Once the plates finished drying, the worms were washed

with 1 mL of M9 before being pipetted into a 1.5 mL centrifuge tube. The tube sat at room temperature for four minutes to allow a pellet to form at the bottom. Extra M9 was pipetted into a waste container. The tube was once again filled with another milliliter of M9 before sitting out for another four minutes to allow the pellet to form again. The excess liquid was discarded into a waste container, with the pellet still in the tube. The pellet was extracted into a 20 uL micropipette before being dropped onto an unseeded NGM plate. The new plate with washed *C. elegans* was taken back into a 16°C room for testing. Once the droplet dried, a capillary tube connected to a mouth tube was used to administer a tiny drop of either the control fluid (diH2O), vape liquid, flavor concentrate, or vape liquid with flavor concentrate at the tail of a forward moving young adult (L4) *C. elegan*. *C. elegans* were not tested more than once. After 4-5 seconds, any avoidance behaviors, determined as at least two backwards reversals or thrashing, were recorded.

RESULTS

4.1 Study 1

4.1.1 Demographics

This study contained data from 48 vapers and 89 non-vapers after cleaning. The predominant demographic groups represented in this sample were White females, followed by White males (Table 1).

Table 1

Demographics of Survey Participants

	Overall		Vapers		Non-vapers	
	N	%	*n*	%	*n*	%
Gender						
Male	47	34.3	15	31.3	32	35.9
Female	81	59.1	32	66.7	49	55.1
Transgender	1	0.73	0	0	1	1.12
Gender fluid	1	0.73	0	0	1	1.12
Non-binary	5	3.65	1	2.08	4	4.49
Prefer not to say	1	0.73	0	0	1	1.12
Other	1	0.73	0	0	1	1.12
Ethnicity						
White	86	62.8	33	68.8	53	59.6
Asian	21	15.3	3	6.25	18	20.2
Pacific Islander	0	0	0	0	0	0
Black or African American	5	3.65	3	6.25	2	2.25
American Indian or AN	0	0	0	0	0	0
Hispanic or Latino/a	5	3.65	1	2.08	4	4.49
Multiple Races	18	13.1	7	14.6	11	12.4
Prefer not to say	2	1.46	1	2.08	1	1.12
Other	0	0	0	0	0	0

Note. Overall N = 137 (Vapers n = 48, Non-vapers n = 89)

4.1.2 Trends in Vapers

In the vaper group, the main motivation behind vaping was due to enjoyment and for leisurely purposes (Figure 4a). They chose to begin vaping either out of curiosity and/or due to peer influences (Figure 4b). Participants mainly reported feeling "less stressed" as an outcome of vaping, followed by feeling happier and like they fit in more with others (Figure 4c). It was found that vapers typically indulge in this behavior largely at parties, indicating that vaping is considered more of a social activity (Figure 4d).

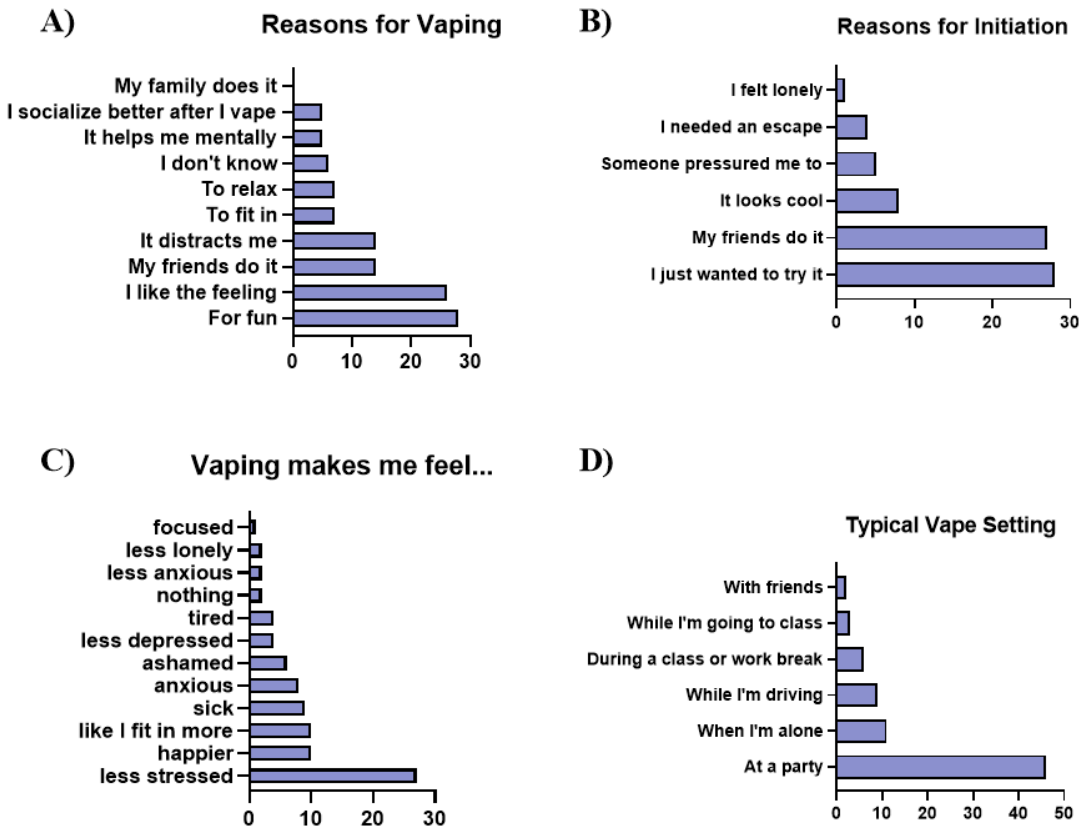

Figure 4. *(a) Reasons why participants (n = 48) choose to vape, (b) why they began vaping, (c) how vaping makes them feel, and (d) the typical setting in which they vape.*

Fruity vape flavors were the most popular vape flavor at 48%, followed by mint at 26%, menthol 16%, and 10% choosing sweet flavors (Figure 5). This is similar to what was found in the literature review, with fruity flavors being the most popular. However, in WPI students, sweet flavored vape juice is the least popular. Some examples of fruity flavored vape flavors provided were watermelon, mango, and blue raspberry, with watermelon being the most common answer. This answer was used to select the flavor concentrate used in Study 2.

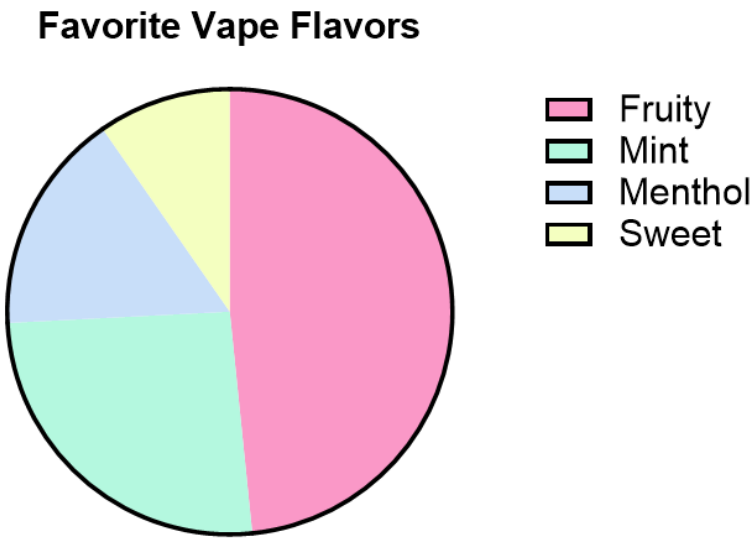

Figure 5. *Participants (n = 48) favorite vape flavor categories.*

A logistic regression was used to analyze whether loneliness, depression, anxiety, or stress could be a predictor of vaping in WPI students (Table 2). There were no significant outcomes, as all the values are above $p = 0.05$. These measures were not found to be psychosocial predictors of vaping.

Table 2

Logistic Regression Analysis Examining Mental Health as a Predictor of Vaping

	B	S.E.	Wald	df	Sig.	Exp(B)
Loneliness	.481	.370	1.687	1	.194	1.618
Depression	-.054	.050	1.191	1	.275	.947
Anxiety	-.049	.055	.812	1	.367	.952
Stress	.232	.504	.211	1	.646	1.261
Constant	.429	.706	.368	1	.544	1.535

4.1.3 Trends in Non-Vapers

For participants that choose not to vape, the number one reasoning was due to health concerns (Figure 6). This could possibly mean that college students are aware of the health complications that arise from vaping, but those who choose to vape may not care about them. A lot of the non-vapers were generally not interested in vaping altogether, and they did not want to get addicted. Vaping was also considered too expensive for participants to be interested in.

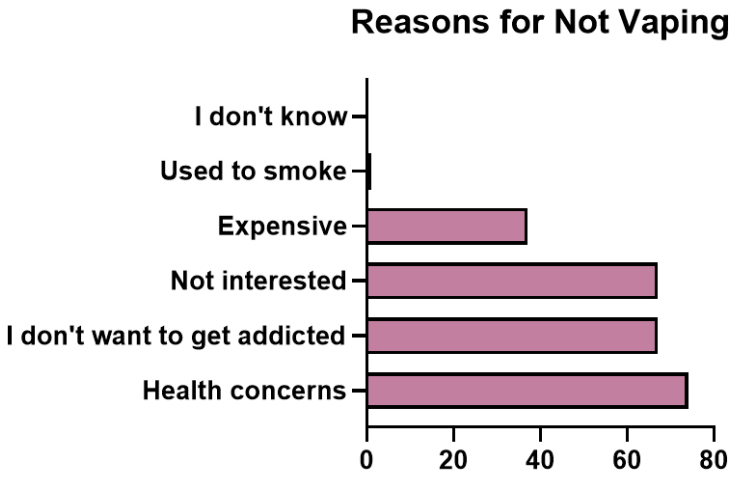

Figure 6. *Reasons why participants (n= 89) choose not to vape.*

4.1.4 Comparisons Between Vapers and Non-Vapers

A correlational analysis was run to examine the relationship between age and depression in both the vaper and non-vaper group. There was a significant, moderate, negative correlation between age and depression in the vaper group, with r = -.319 and p = .026. There was a slight negative correlation (r = -.149) between age and depression in non-vapers, however, it was not statistically significant (p = .169). The relationship between age and depression in vapers indicates that younger vapers tend to experience higher depressive symptoms.

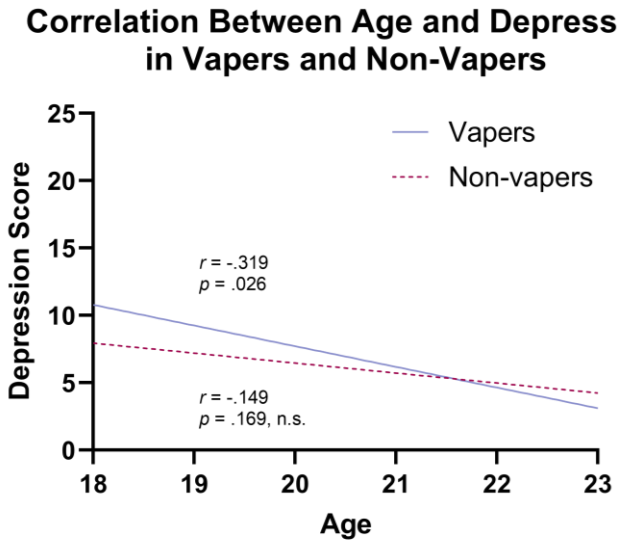

Figure 7. *Correlation analysis examining the relationship between age and depression in vapers and non-vapers.*

Figure 8 displays and compares the average scores of each mental health measure in the vaper and non-vaper groups. None of the differences between the groups were found to be statistically significant. However, it is clear that vapers scored slightly higher on the PHQ8 and GAD-7 questionnaires compared to the non-vapers. The non-vapers scored slightly higher on the

UCLA Loneliness Scale than the vapers – another indication that vaping may be more of a social activity than a psychological outcome. Both groups scored similarly on the Perceived Stress Scale.

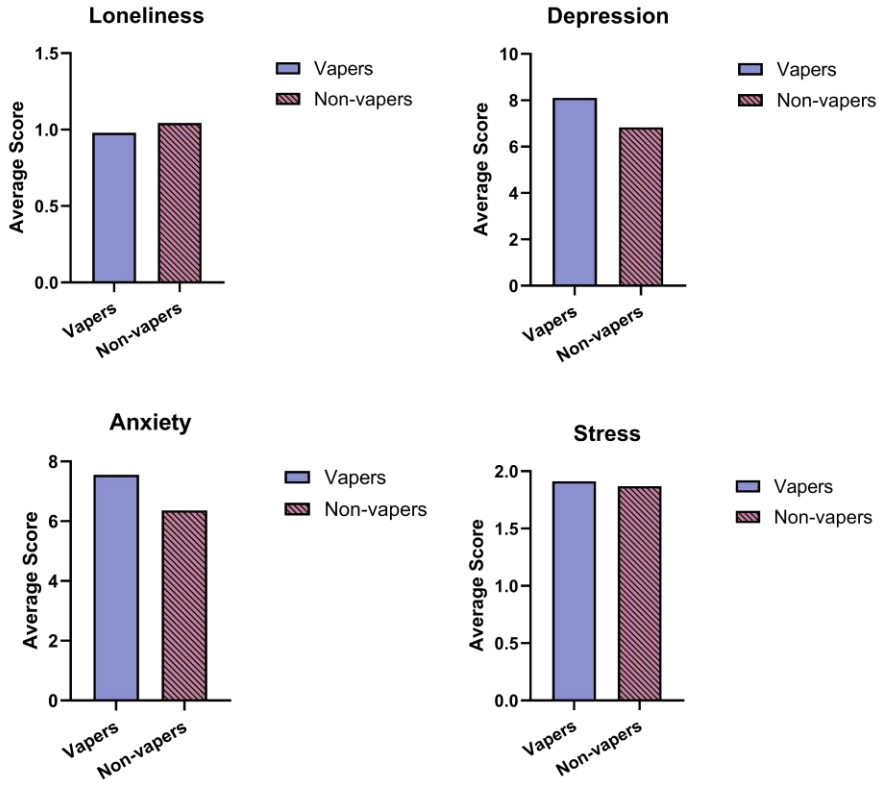

Figure 8. *Comparisons of average scores of the mental health measures between vapers and non-vapers. None of the differences are statistically significant.*

4.2 Study 2

240 *C. elegans* were subjected to a tiny drop of either diH_2O, flavor concentrate, nicotine, or nicotine with flavoring and avoidance behaviors (at least two backward reversals) were recorded. A t-test was run to compare the 5% and 10% nicotine condition. There was a significant increase in the avoidance index when the percentage of nicotine jumped from 5 to 10 percent, with $p = 0.044$. There were no significant differences between the 10 and 15 percent

nicotine groups, and no significant differences between the nicotine and nicotine with flavoring conditions. These results show that *C. elegans* do avoid nicotine at increasing concentrations, but it is possible that there is a certain threshold. Higher concentrations above 15 percent would have to be tested to observe if there could possibly be a 100% avoidance rate.

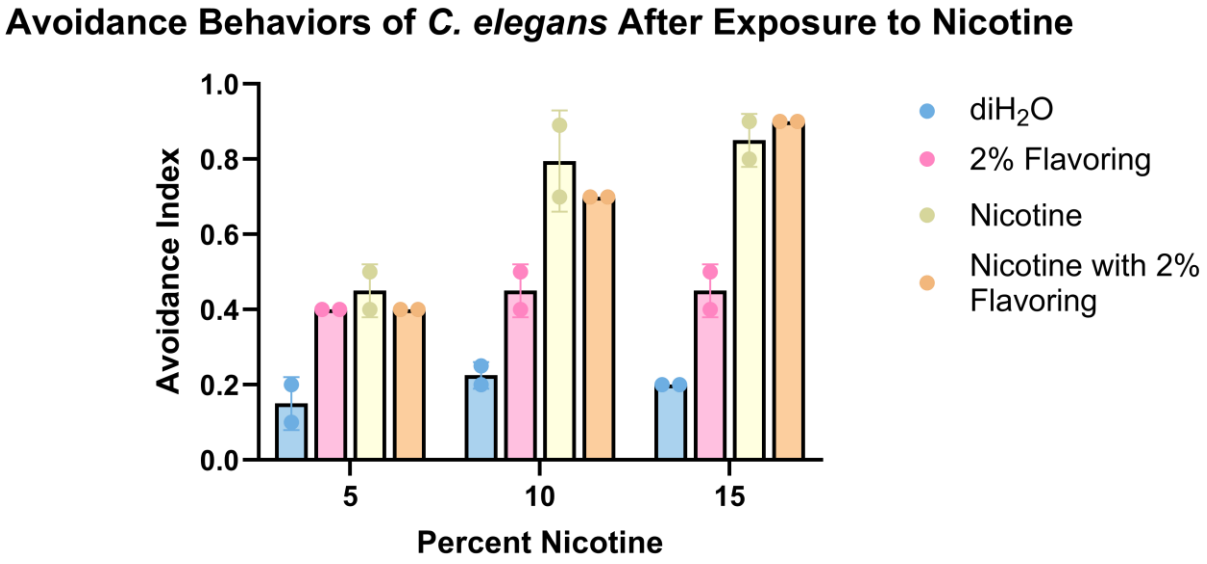

Figure 9. *Avoidance index of C. elegans after being exposed to varying concentrations of nicotine. The diH_2O and 2% flavoring conditions contained no nicotine. Avoidance index was calculated by dividing the number of drops avoided by the total drops administered.*

DISCUSSION

Across two complementary studies, it was found that vaping appears to be a social activity among WPI students rather than an outcome from psychological mood states, though nicotine can cause behavioral consequences at the biological level.

Younger Vapers Have More Depressive Symptoms at WPI

At WPI, it was found that younger vapers scored higher on the PHQ8. There are many possible factors that may account for this relationship. One explanation is that younger college students may have been exposed to vaping at a younger age, and nicotine has been shown to increase odds of depression. It is also possible that social media influenced these younger vapers, as following trends to be considered "cool" and to "fit in" is common in this current generation. However, this study did not investigate social media as a driver of vaping behavior and future work should investigate this further.

Vaping is a Social Activity at WPI

Loneliness, depression, anxiety, and stress were not found to be predictors of vaping in WPI students. Instead, a large portion of the participants reported that they began vaping because their friends do it, and they mainly vape at parties. Vapers scored somewhat lower than non-vapers on the loneliness scale, suggesting that they may feel a little more social fulfillment than non-vapers. However, this difference was non-significant. Some participants reported that they feel like they fit in more when they vape and that they continue to vape because their friends do it. It seems that, overall, vaping at WPI is heavily peer influenced rather than a possible coping mechanism.

C. elegans Exhibit Avoidance Behavior to Increasing Concentrations of Nicotine

When the amount of nicotine changed from 5 to 10 percent, there was a significant increase in avoidance. *C. elegans* find nicotine to be an aversive stimulus at higher concentrations. With *C. elegans* being a model organism for humans, this is supporting evidence that higher concentrations of nicotine are bad for the human body as well.

5.1 Strengths and Limitations

This is the first interdisciplinary study with a comparative component that examined vaping behavior. It also examined these vaping behaviors in a relevant population (college students) and developed questionnaires based on conversations with probing lived experience in this population, which supports strong construct validity across the items. This project also utilized survey results to identify the most popular vape flavor at WPI, and this informed on the choice of flavoring to incorporate into experimentation with *C. elegans*. In this way, the studies were not only comparative, but also synergistic. Results from this study can be used to understand why vaping occurs at WPI and inspire administration to be proactive in discouraging vaping, especially in social situations.

It is important to note, however, that the survey sample lacked demographic diversity. While it may be somewhat representative of the general population of WPI, it is nonetheless important to examine these behaviors in students with a range of sociodemographic backgrounds. Additionally, the survey was administered to a relatively small sub-group of the WPI population. That is, only those taking a Psychology course were present in the subject pool. While the subject pool typically contains a wide range of students in terms of graduation year and major, there are nonetheless many students who never take a Psychology course and therefore would not be represented in this sample. Some participants did not understand that the survey was looking for habitual vapers and not those who have only vaped once or twice in their lifetime. For Study 2, only one behavioral assay was conducted – this work should be continued with further experimentation with different conditions and assays.

5.2 Future Directions

To widen and diversify the sample size, it would be important to send out this survey to all WPI undergraduates and other students at different colleges across the country. The survey would have to be re-worded to be catered toward habitual vapers so more accurate responses can be provided. If the sample size were to be larger and more representative, it would be possible to draw more generalizable conclusions.

Several behavioral assays should be conducted in the future. Egg-laying assays have been used in nicotine exposure research previously and should be conducted with the same conditions and groups used in the avoidance assays in this project. It would also be important to conduct chemotaxis assays to further understand how nicotine affects *C. elegans* chemo-sensation and avoidant behaviors. Repeating the avoidance assays with different concentrations of nicotine between 5 and 10 percent to see when the avoidance index jump happens would be beneficial to the understanding of when nicotine begins to be noticeably harmful.

5.3 Conclusion

With the rise of e-cigarettes and the high levels of vaping in college-aged adults, it is increasingly important to understand the implications of vaping both psychologically and biologically in this age-group. This project represents an important foundational step in advancing the goal of addressing these knowledge gaps. While vaping may be primarily driven by social influence in college students, there are nonetheless biobehavioral consequences of nicotine use, which was elucidated through experimentation in the model organism, *C. elegans*. Future research should continue to capitalize on the comparative research paradigms to fully investigate both the social and biological implications of vaping and nicotine use.

APPENDICES

Appendix A: Vaping Survey

1. How old are you?
2. What ethnicity are you? (Select all that apply)
    a. White
    b. Asian
    c. Pacific Islander
    d. Black or African American
    e. American Indian or Alaska Native
    f. Hispanic or Latino/a
    g. Multiple Races
    h. Prefer not to say
    i. Other (please specify)
3. What gender do you identify as?
    a. Male
    b. Female
    c. Transgender
    d. Gender fluid
    e. Non-binary
    f. Prefer not to say
    g. Other (please specify)
4. What is your planned graduation year?
    a. 2024

   b. 2025
   c. 2026
   d. 2027
   e. Other

5. If you selected “Other” for your planned graduation year, please specify the year below.
6. Have you ever vaped?
   a. Yes
   b. No
7. What is your general opinion of vaping?
   a. Extremely negative
   b. Somewhat negative
   c. Neither positive nor negative
   d. Somewhat positive
   e. Extremely positive
8. Do your friends vape?
   a. Yes
   b. No
   c. I don’t know
9. How many of your friends vape?
   a. None of my friends
   b. Some of my friends
   c. Most of my friends
   d. I don’t know

If "Yes" was selected in Q6…

1. What concentration(s) have you vaped in the past (in mg)? Select all that apply.
    a. 3-5 mg
    b. 6-11 mg
    c. 12-18 mg
    d. 20+ mg
    e. I don't know
    f. Other (please specify)
2. Do you own a vape pen?
    a. Yes
    b. No
3. How often do you vape on average?
    a. Once a month
    b. Multiple times a month
    c. Once a week
    d. Multiple times a week
    e. Once a day
    f. Multiple times a day
    g. Other (please specify)
4. What concentrations do you typically vape (in mg)?
    a. 3-5 mg
    b. 6-11 mg

c. 12-18 mg

d. 20+ mg

e. I don’t know

f. Other (please specify)

5. During which occasions or situations do you typically vape? (Select all that apply)

a. At a party

b. When I’m alone

c. While I’m driving

d. While I’m going to class

e. During a class or work break

f. Other (please specify)

6. Why do you vape? (Select all that apply)

a. I like the feeling

b. To fit in

c. My friends do it

d. My family does it

e. It helps me mentally

f. It distracts me

g. For fun

h. I socialize better after I vape

i. To relax

j. I don’t know

k. Other (please specify)

7. What is your favorite vape flavor?
8. What brand of vape do you use?
9. When did you start vaping?
    a. Before middle school
    b. During middle school
    c. Before high school
    d. During high school
    e. During college
10. What led you to begin vaping? (Select all that apply)
    a. My friends do it
    b. Someone pressured me to
    c. It looks cool
    d. I just wanted to try it
    e. I needed an escape
    f. I felt lonely
    g. Other (please specify)
11. Do you typically vape alone or around other people?
    a. Alone
    b. With other people
    c. Both
12. Vaping makes me feel… (Select all that apply)
    a. Less stressed
    b. Sick

c. Happier

d. Like I fit in more

e. Ashamed

f. Less lonely

g. Anxious

h. Less depressed

i. Tired

j. Other (please specify)

If "No" was selected in Q6…

1. Have you ever considered vaping?
    a. Yes
    b. No
2. Have you ever felt pressured to start vaping?
    a. Yes
    b. No
3. Why do you choose not to vape? (Select all that apply)
    a. Health concerns
    b. I don't want to get addicted
    c. Not interested
    d. Expensive
    e. I don't know
    f. Other (please specify)

Appendix B: UCLA Loneliness Scale

Indicate how often each of the statements below is descriptive of you.

I am unhappy doing so many things alone	Never	Rarely	Sometimes	Often
I have nobody to talk to	Never	Rarely	Sometimes	Often
I cannot tolerate being so alone	Never	Rarely	Sometimes	Often
I lack companionship	Never	Rarely	Sometimes	Often
I feel as if nobody really understands me	Never	Rarely	Sometimes	Often
I find myself waiting for people to call or write	Never	Rarely	Sometimes	Often
There is no one I can turn to	Never	Rarely	Sometimes	Often
I am no longer close to anyone	Never	Rarely	Sometimes	Often
My interests and ideas are not shared by those around me	Never	Rarely	Sometimes	Often
I feel left out	Never	Rarely	Sometimes	Often
I feel completely alone	Never	Rarely	Sometimes	Often
I am unable to reach out and communicate with those around me	Never	Rarely	Sometimes	Often
My social relationships are superficial	Never	Rarely	Sometimes	Often
I feel starved for company	Never	Rarely	Sometimes	Often

No one really knows me well	Never	Rarely	Sometimes	Often
I feel isolated from others	Never	Rarely	Sometimes	Often
I am unhappy being so withdrawn	Never	Rarely	Sometimes	Often
It is difficult for me to make friends	Never	Rarely	Sometimes	Often
I feel shut out and excluded from others	Never	Rarely	Sometimes	Often
People are around me but not with me	Never	Rarely	Sometimes	Often

Appendix C: PHQ8

Over the last 2 weeks, how often have you been bothered by any of the following problems?

Little interest or pleasure in doing things	Not at all	Several days	More than half the days	Nearly every day
Feeling down, depressed, or hopeless	Not at all	Several days	More than half the days	Nearly every day
Trouble falling asleep or staying asleep, or sleeping too much	Not at all	Several days	More than half the days	Nearly every day
Feeling tired or having little energy	Not at all	Several days	More than half the days	Nearly every day

Poor appetite or overeating	Not at all	Several days	More than half the days	Nearly every day
Feeling bad about yourself – or that you are a failure and have let yourself or your family down	Not at all	Several days	More than half the days	Nearly every day
Trouble concentrating on things, such as reading the newspaper or watching television	Not at all	Several days	More than half the days	Nearly every day
Moving or speaking so slowly that other people could have noticed. Or the opposite – being so fidgety or restless that you have been moving around a lot more than usual	Not at all	Several days	More than half the days	Nearly every day

Appendix D: GAD-7

Over the last 2 weeks, how often have you been bothered by any of the following problems?

Feeling nervous, anxious, or on edge	Not at all	Several days	More than half the days	Nearly every day
Not being able to stop or control worrying	Not at all	Several days	More than half the days	Nearly every day
Worrying too much about different things	Not at all	Several days	More than half the days	Nearly every day
Trouble relaxing	Not at all	Several days	More than half the days	Nearly every day
Being so restless that it is hard to sit still	Not at all	Several days	More than half the days	Nearly every day
Becoming easily annoyed or irritable	Not at all	Several days	More than half the days	Nearly every day
Feeling afraid, as if something awful might happen	Not at all	Several days	More than half the days	Nearly every day

Appendix E: Perceived Stress Scale

In the last month, how often have you…

…been upset because of something that happened unexpectedly	Never	Almost never	Sometimes	Fairly often	Often

…felt that you were unable to control the important things in your life?	Never	Almost never	Sometimes	Fairly often	Often
…felt nervous and stressed?	Never	Almost never	Sometimes	Fairly often	Often
…dealt successfully with irritating life hassles?	Never	Almost never	Sometimes	Fairly often	Often
…felt that you were effectively coping with important changes that were occurring in your life?	Never	Almost never	Sometimes	Fairly often	Often
…felt confident about your ability to handle your personal problems?	Never	Almost never	Sometimes	Fairly often	Often
…felt that things were going your way?	Never	Almost never	Sometimes	Fairly often	Often
…found that you could not cope with all the things you had to do	Never	Almost never	Sometimes	Fairly often	Often
…been able to control irritations in your life?	Never	Almost never	Sometimes	Fairly often	Often
…felt that you were on top of things?	Never	Almost never	Sometimes	Fairly often	Often

…been angered because of things that happened that were outside your control?	Never	Almost never	Sometimes	Fairly often	Often
…found yourself thinking about things that you had to accomplish?	Never	Almost never	Sometimes	Fairly often	Often
…been able to control the way you spend your time?	Never	Almost never	Sometimes	Fairly often	Often
…felt difficulties were piling up so high that you could not overcome them?	Never	Almost never	Sometimes	Fairly often	Often

REFERENCES

1. Dinardo, P., & Rome, E. S. (2019). Vaping: The new wave of nicotine addiction. *Cleveland Clinic journal of medicine*, *86*(12), 789-798.
2. Kramarow, E. A., & Elgaddal, N. (2023). Current electronic cigarette use among adults aged 18 and over: United States, 2021.
3. Mishra, A., Chaturvedi, P., Datta, S., Sinukumar, S., Joshi, P., & Garg, A. (2015). Harmful effects of nicotine. *Indian Journal of Medical and Paediatric Oncology*, *36*(01), 24-31.
4. Bility, M. M. T. (2019). Are rises in the Lithosphere-Magnetic Field in the United States, interacting with vaping aerosols-iron in lungs, the tipping point for the outbreak of vaping-associated acute lung injury.
5. Sinharoy, P., McAllister, S. L., Vasu, M., & Gross, E. R. (2019). Environmental aldehyde sources and the health implications of exposure. *Aldehyde dehydrogenases: From alcohol metabolism to human health and precision medicine*, 35-52.
6. Simon, L., Emery, B., & Carrell, D. T. (2019). Sperm DNA fragmentation: consequences for reproduction. *Genetic Damage in Human Spermatozoa*, 87-105.Simon, L., Emery, B., & Carrell, D. T. (2019). Sperm DNA fragmentation: consequences for reproduction. *Genetic Damage in Human Spermatozoa*, 87-105.
7. Tobore, T. O. (2019). On the potential harmful effects of E-Cigarettes (EC) on the developing brain: The relationship between vaping-induced oxidative stress and adolescent/young adults social maladjustment. *Journal of adolescence*, *76*, 202-209.

8. Gorfinkel, L., Hasin, D., Miech, R., & Keyes, K. M. (2022). The link between depressive symptoms and vaping nicotine in US adolescents, 2017–2019. *Journal of Adolescent Health*, *70*(1), 133-139.
9. Dubé, C. E., Pbert, L., Nagawa, C. S., Simone, D. P., Wijesundara, J. G., & Sadasivam, R. S. (2023). Adolescents who vape nicotine and their experiences vaping: a qualitative study. *Substance Abuse: Research and Treatment*, *17*, 11782218231183934.
10. Leslie, F. M. (2020). Unique, long-term effects of nicotine on adolescent brain. *Pharmacology Biochemistry and Behavior*, *197*, 173010.
11. Balfour, D. J., & Ridley, D. L. (2000). The effects of nicotine on neural pathways implicated in depression: a factor in nicotine addiction?. *Pharmacology Biochemistry and Behavior*, *66*(1), 79-85.
12. Gurusamy, R., & Natarajan, S. (2013). Current status on biochemistry and molecular biology of microbial degradation of nicotine. *The Scientific World Journal*, *2013*.
13. Mishra, A., Chaturvedi, P., Datta, S., Sinukumar, S., Joshi, P., & Garg, A. (2015). Harmful effects of nicotine. *Indian Journal of Medical and Paediatric Oncology*, *36*(01), 24-31.
14. Ortells, M. O., & Barrantes, G. E. (2010). Tobacco addiction: a biochemical model of nicotine dependence. *Medical hypotheses*, *74*(5), 884-894.
15. Oliver, A. L., Kossowsky, J., Minegishi, M., Levy, S., & Weitzman, E. R. (2023). The Association of Vaping With Social/Emotional Health and Attitudes Toward COVID-19 Mitigation Measures in Adolescent and Young Adult Cohorts During the COVID-19 Pandemic. *Substance abuse*, *44*(1), 73-85.

16. Dyal, S. R., & Valente, T. W. (2015). A systematic review of loneliness and smoking: small effects, big implications. *Substance use & misuse*, *50*(13), 1697-1716.

17. Zhang, Z., Mei, X., He, Z., Xie, X., Yang, Y., Mei, C., ... & Zhong, W. (2022). Nicotine metabolism pathway in bacteria: mechanism, modification, and application. *Applied Microbiology and Biotechnology*, *106*(3), 889-904.

18. Kaletta, T., & Hengartner, M. O. (2006). Finding function in novel targets: C. elegans as a model organism. *Nature reviews Drug discovery*, *5*(5), 387-399.

19. Feng, Z., Li, W., Ward, A., Piggott, B. J., Larkspur, E. R., Sternberg, P. W., & Xu, X. S. (2006). A C. elegans model of nicotine-dependent behavior: regulation by TRP-family channels. *Cell*, *127*(3), 621-633.

20. Kudelska, M. M., Lewis, A., Ng, C. T., Doyle, D. A., Holden-Dye, L., O'Connor, V. M., & Walker, R. J. (2018). Investigation of feeding behaviour in C. elegans reveals distinct pharmacological and antibacterial effects of nicotine. *Invertebrate Neuroscience*, *18*, 1-8.

21. Polli, J. R., Dobbins, D. L., Kobet, R. A., Farwell, M. A., Zhang, B., Lee, M. H., & Pan, X. (2015). Drug-dependent behaviors and nicotinic acetylcholine receptor expressions in Caenorhabditis elegans following chronic nicotine exposure. *Neurotoxicology*, *47*, 27-36.

22. Zou, W., Cheng, H., Li, S., Yue, X., Xue, Y., Chen, S., & Kang, L. (2017). Polymodal responses in C. elegans phasmid neurons rely on multiple intracellular and intercellular signaling pathways. *Scientific reports*, *7*(1), 42295.

23. Hilliard, M. A., Bargmann, C. I., & Bazzicalupo, P. (2002). C. elegans responds to chemical repellents by integrating sensory inputs from the head and the tail. *Current Biology*, *12*(9), 730-734.

24. Maruyama, I. N. (2017). Receptor guanylyl cyclases in sensory processing. *Frontiers in endocrinology*, *7*, 173.

25. WormAtlas, Altun, Z.F., Herndon, L.A., Wolkow, C.A., Crocker, C., Lints, R. and Hall, D.H. (ed.s) 2002-2024. http://www.wormatlas.org

26. Russell, D , Peplau, L. A.. & Ferguson, M. L. (1978). Developing a measure of loneliness. Journal of Personality Assessment, 42, 290-294

27. Kroenke, K., Spitzer, R. L., & Williams, J. B. (2001). The PHQ-9: validity of a brief depression severity measure. *Journal of general internal medicine*, *16*(9), 606-613.

28. Spitzer, R. L., Kroenke, K., Williams, J. B., & Löwe, B. (2006). A brief measure for assessing generalized anxiety disorder: the GAD-7. *Archives of internal medicine*, *166*(10), 1092-1097.

29. Cohen, S., Kamarck, T., & Mermelstein, R. (1983). A global measure of perceived stress. Journal of Health & Social Behavior, 24(4), 385–396.