LONG TERM GRANT APPLICATION
Cover Sheet

Amount Requested: $3,499.96

Project Information

Marston Clarissa

Student Participant (Last, First)

The Effects of Vaporized Nicotine Dose and Puff duration on Locomotor Activity in Mice

Project Title (10 words or less)

Hillhouse, Todd 1202

Faculty Mentor Name (last, first) Mail Code

College of Social and Behavioral Science Psychology

College (Weber State is the University, NOT college) Department

This project _X_ DOES/___ DOES NOT require review by the WSU Institutional Review Board for Human Subjects or the WSU Animal Care and Use Committee.

Clarissa Marston
Student Signature

1-4-19
Date

Project Mentor Signature

1/15/19
Date Received by Mentor.

1202
Campus Mail

6315
Phone Ext.

Undergraduate Research Committee Representative

28 January 2019
Date Received by URC Rep.

Faculty Mentor Department Chair

31 Jan 2019
Date

Please check if attended Research Proposal Workshop:

[X] Date Workshop attended October 24th 2018 (Please fill in the date of attendance)

Jennifer B. Howell 1/31/19

LONG TERM GRANT APPLICATION
Budget Worksheet

BUDGET ITEM	Department or College Funds	Dr. Hillhouse Research Funds	Personal Funds	Undergrad. Research Funds	GRAND TOTAL
Materials	Neuroscience Program and CSBS Deans office will contribute $50 each ($100 total) for lab consumables/ unforeseen expenses.	50 mg of mecamylamine = $399 Lab consumables = $250 E-juice/nicotine = ~$500 (will purchase larger quantity, but this project will use the amount above)		72 mice (36 male and 36 female) with shipping = $2,355.50 50 mg of Dihydro-β-erythroidine hydrobromide = $815 Animal Husbandry cost = $129.46	$4,548.96
Equipment		Locomotor motor activity chambers and software (in kind) e-Vape Delivery system (in kind)			
Research Scholarship (max request $2,500.00)				Research Stipend = $200	$200.00
Mileage to gather Data (.38 per mile)					
GRAND TOTAL	$100.00	$1,149.00		$3,499.96	$4,748.96

NOTES:

Project Description

Research Question: How does vaporized nicotine dose, vape session length, and gender influence behavioral and physiological changes in mice?

From their introduction in the United States in 2007, electronic nicotine delivery systems (ENDS) have rapidly established an ever-growing consumer base. ENDS products include e-cigarettes and newer products such as JUUL, Mod, Vuse, etc. ENDS usage is higher than ever for Americans aged 12-18 with approximately 20.8% of teenagers using, which is a 78% increase from the year 2017 to 2018 (Cullen, 2011-2018). Additionally, the number of working Americans who are ENDS users has increased to approximately 3.8% (5.5 million people) (Syamlal, 2014).

Prior research has been conducted to understand the behavioral implications of nicotine in users of traditional combustible cigarettes. Cigarettes contain a set dose of nicotine ranging from 6 -18 mg per cigarette depending on the strength of cigarette. Interestingly, the user only received 1-2 mg of nicotine when using a regular strength cigarettes because of the combustible nature of the cigarette (e.g. the cigarette continues to burn even when the users in not inhaling). The known and controlled amount of nicotine in cigarettes has allowed researchers to establish a relationship between nicotine dose and behavioral changes, which is not the case with ENDS. In contrast to traditional cigarettes, ENDS users select their nicotine dose a variety of nicotine concentrations (0-30.0 mg/mL). Furthermore, vape session lengths are also left up to the discretion of the user as there is not a combustible aspect to ENDS, which allows users to take one puff or multiple vapes at a time with little wasted nicotine. ENDS users typically vape periodically throughout the day. Taken together, the variety of variables within ENDS usage (i.e. puff duration, nicotine concentration, vape session length, etc.) make it difficult to calculate how much nicotine an individual is exposed to and determine the relationship between vaporized nicotine dose and behavior changes.

Current preclinical research is restricted in terms evaluating puff duration, nicotine concentration, and vape session length of vaporized nicotine on behavior. For example, one study used a single dose of nicotine at 16.8 mg/mL daily administered via ENDS for 30 minute sessions, with puffs occuring 25 per minute, over the course of 7 weeks (Ponzoni, 2015). Another study administered doses of either 0, 12, 24, or 30 mg/mL in 1 puff lasting 10 seconds and then mice were held in the vape chamber for 1 minute before being returned to their home cages for 2 minutes. This process was repeated 5 times in a 13 minute session (Lefever, 2017). In particular, there is a lack of research devoted to understanding the changes of locomotor behaviors and body temperature with respect to both designated nicotine concentrations and session lengths. The aim of this study is to: 1) Establish a vaporized nicotine dose response curve (0-30 mg/mL) for a 10 min session in mice. 2) Evaluate how vape session length (10 min vs 30 min) changes the behavioral effects of vaporized nicotine. 3) Use nicotine antagonist drug pretreatment to determine that all behavioral effects following vape session are mediated by nicotine and not a side effect of inhaling vapor. Additionally, all study will be perform in male and female mice which will allow us to evaluate gender differences.

I have selected Dr. Todd Hillhouse to be my mentor for this project based on his experience in the field of behavioral pharmacology. Dr. Hillhouse will advise me as the primary investigator on the proposed studies. He will also provide initial training on using the vape administration system and the locomotor boxes. As the primary investigator for this project, I will be running the experiments, analyzing the results and preparing findings for dissemination.

Dependent _____X____ Independent

(student helping faculty do research) (student doing own research)

In order to cultivate my understanding of this research project I have taken the following courses: introduction to neuroscience, psychology of drug addiction, human biology, and cellular and molecular neuroscience. I am currently enrolled in biopsychology and research methods and stats II.

Additionally, I have been working in Dr. Hillhouse's animal lab on other studies involving drug administration since summer 2018 semester. Lastly, I have worked on several clinical research projects for Dr. Hillhouse and the Psy Chi club. I plan to present the findings from this study at the American Society for Pharmacology and Experimental Therapeutics Conference in April 2020, WSU research symposium in Spring 2020, and submit the findings for publication in peer reviewed journals (e.g. *Drug and Alcohol Dependence* or *Behavioural pharmacology)*.

Project Methods & Timeline

Spring 2019: My current study has already received approval from the Institutional Animal Care and Use Committee (IACUC) and data collection sessions are scheduled to begin during the Spring 2019 semester. A total of 72 mice will be needed to complete all phases of the proposed research. Eighteen mice will be ordered in spring for the first phase of the study in which half will be males and half females. I will start 10 minute vape sessions with a 3 second puff duration occurring every two minutes on three groups containing three mice each. Body temperature will be recorded before and after each dose of nicotine to examine possible hypothermic effects of the drug. Mice will be placed in a locomotor activity (LMA) box with IR sensors to track their behavior for 60 minutes. Locomotor activity is a basic measure of behavior that can show bidirectional change in activity levels (increases and decreases in behavior. We will use five doses of nicotine to establish a dose response curve (0-30.0 mg/mL). We will use a within subjects design in which each mouse will received all doses of nicotine. Each mouse will receive one dose per LMA session (maximum of two doses per week).

Summer 2019: By the first few weeks of the summer semester I believe that I will have finished enough data collection to complete a dose response curve. I will use this information to determine options for further testing. I will evaluate the same dose response curve (0-30.0 mg/mL) for a 30 minute vape session (order 18 mice), which will allow me to compare the behavioral effects of nicotine for both 10 and 30 min vape sessions.

Fall 2019: During the fall semester, I will conduct two antagonist studies to determine the receptors responsible for vaporized nicotine behavior, in which the dosing and session lengths will be based on my results from the previous two semesters/studies. First, we will evaluate the ability of the nonspecific nicotinic receptor antagonist, mecamylamine, to block the behavioral and physiological effects of vaporized nicotine (order 18 mice). Next, we will determine if the behavioral effects of vaporized nicotine are mediated by the α4β2 receptors by using a selective α4β2 receptor antagonist, Dihydro-β-erythroidine hydrobromide (order 18 mice). The α4β2 receptors are thought to play a role in memory, increased mood, and psychological withdrawal.

Budget Explanation

I am requesting funds to purchase 72 C57BL/6 mice (36 male and 36 female) from Charles River Laboratories that totals $2,355.50. This number of mice are required to maintain statistical power for these experiments. I am requesting $129.46 to supply food and bedding for the mice. I need to purchase the selective α4β2 Dihydro-β-erythroidine hydrobromide for the antagonist study ($815). I am requesting a $200.00 research scholarship stipend to allow me to take time off work to run this project. I will be working approximately 10 hours a week on research project for the duration of the research project. See appendix for quotes on all items.

Dr. Hillhouse will purchase mecamylamine ($399), E-juice and nicotine (~$500), and lab consumables (syringes, gloves, heap filters, etc.; $250) specifically for this research project. Additionally, Dr. Hillhouse will provide animal housing (i.e. cages, water bottles, cage tops, etc.) and testing equipment (i.e. E-vape delivery system, locomotor chambers, thermometer). Lastly, the Neuroscience Program and College of Social and Behavioral Sciences Deans Office will contribute $50 each (total $100) for lab consumables or unforeseen cost. Dr. Hillhouse will cover any additionally expenses that might come up (e.g. additional mice, different drugs, etc.)

For references, see Appendix A.

LONG TERM GRANT APPLICATION
Additional Questions

1. What funding have you received from OUR in the past? Where has your previous project been disseminated?

   I have not received OUR grant funding in the past.

2. Is this project part of a required course? If so, please indicate the support (monetary and in-kind) provided for this project by the academic department.

   This project is not a required course. However, I am enrolled in the course Projects and Research (NEUR 4800) with Dr. Hillhouse this semester for working on various projects in his lab (not the proposed project in this grant). NEUR 4800 is designed to give students the opportunity to peruse their own research outside of a classroom setting with instructor supervision. It provides valuable hands on experience and skill development that can be translated to future research projects, which has allowed me to propose the current project.

3. What additional sources of funding have been solicited? Is your department willing/able to fund any equipment they will be retaining?

   Dr. Hillhouse ($1,149), the Neuroscience Program ($50), and the CSBS Deans office ($50) will all contribute funds for the project. However, these funds are not enough to fund the entire project. Additionally, Dr. Hillhouse is contribution the behavioral equipment and housing for this study. The largest expense are mice which makes up the majority of the cost associated with this OUR grant.

4. Where do you plan to disseminate the results of this project?

   I will also be submitting this project to the American Society for Pharmacology and Experimental Therapeutics conference in April 2020. Additionally, I will present the results from this project at WSU research symposium in Spring 2020. I intend to work with Dr. Hillhouse on producing a paper for publication in a peer reviewed journal.

5. If you are requesting a Research Scholarship, please list all significant time commitments (5+ hours per week) that you expect to maintain over the duration of your project including, for example, class and work schedules.

   Each session of locomotor activity tracking will span over about 4 hours which will include dosing the mice with nicotine vapor, measuring temperatures before and after dosing, collecting weight data, and a 60-minute tracking session. These sessions will happen twice a week for a total of 8 hours per week. I will also be in the lab 2-3 hours per week to help maintain the cleanliness, check controls, and change cages. Together I will work approximately 11 hours per week on this project.

LONG TERM GRANT APPLICATION
Faculty Recommendation Form

Student Name (last, first): Marston, Clarissa

Project Title: Effects of Vaporized Nicotine on Locomotor Activity in Mice

Mentor Directions: After carefully reviewing the proposal and assessing both the viability of this project and the qualifications of the student requesting funding, answer the questions found below. Please expand the sections as necessary (**do not attach separate letter**). If the project involves the use of human subjects or protected animals, be sure the student secures IRB or ACUC approval. If the project receives funding, it is your responsibility to work closely with the student, monitor the ongoing progress of the project and budget, and evaluate the project's results. Failure to do so will jeopardize funding for this project and any future projects.

1. How long and in what capacity have you known this student?
I have known Clarissa Marston since Fall 2016. She was enrolled in my Introduction to Psychology course. She works at the computer lab in the Social and Behavioral Science building and we continue to discuss career goals after the Intro to Psychology course. She expressed interest in neuroscience so I asked her to work on a clinical research project that was lead by McKenzie Peterson. Clarissa excelled on this project and took over as lead investigator when McKenzie graduated. Clarissa then moved to the animal lab once we set that up in the summer of 2018. She has worked on several small project over the past 6 months and is excelling in this line of research.

2. Briefly describe the proposed project. Is this part of a larger research project? Is this part of a course? If so, how is the project apart from the nature and scope of activities normally taken for the course (Please attach a copy of your course syllabus)?
Clarissa is going to evaluate how nicotine dose, vape session length, and gender change the behavioral and physiological effects in mice. Additionally, she will evaluate what nicotinic receptors are responsible for behavioral changes in mice. This study will provide evidence to the valuable information to scientist in the tobacco/nicotine fields. I have an interest in ENDS and vaping, but this will be my first project to evaluate the effects of vaping in mice. Clarissa designed the studies with my guidance. My goal will be to expand on this project if successful. This project is NOT part of a course. Clarissa is currently enrolled in NEUR 4800 for other research projects and may sign up for NEUR 4800 in the fall to allow her to work on this project.

3. Give an assessment of the project's significance to the student's discipline and of the project's educational and/or professional benefit to the student.
The faculty in the Psychology Department and Neuroscience Program feel that research understanding is fundamental to a students understand of the science of psychology. As such, contextually grounded hands on experience on a research project is the best way for a student to gain that knowledge. As part of this project, Clarissa will better develop a working understanding of psychological and neuroscience research. Additionally, the experience she will gain will be incredibly valuable in his continuing professional development as he plans to apply for graduate school. Clarissa has the goal to attend graduate school for Neuroscience, and I strongly believe an independent project like this enhance her application.

4. Comment on the qualifications of the student to successfully complete this project, both in terms of the project's scope and its time frame.
Clarissa has been a valuable student both in the classroom and the research lab. She has impressed me with her intelligence, determination, and writing abilities. I have no doubt in my mind that Clarissa can and will complete this project as she took over lead investigator on a different project and completed that project. We are currently writing the manuscript for that research project, which will be submitted by the end of the spring semester (she is second author). The time frame for this project will allow for her to complete the project. As mentioned above, I

have significant area in this research and understand what it takes to complete this type of project. I strongly believe that Clarissa displays all of the skills and mindset required to complete an independent research project on time. She will be able to present her research findings at neuroscience and pharmacology conferences as well as submit the results for publication in a peer review journal.

5. Comment on the justification and appropriateness of the project budget, including the necessity of a Research Scholarship (if requesting one).
Clarissa is primarily requesting funds to order animals for this project, which is the largest expense ($2355.50). She is also requesting funds to order an antagonists and food and bedding for the mice ($944.46). I have committed $1,149 of my research funds to purchase lab consumables, E-juice/nicotine, and mecamylamine. We have provide a quote for nicotine which is more than the $500 indicated here because I will be ordering a larger quantity (1 gram) to save money. We will use approximately 500 mg of nicotine for this study (E-juice quote is attached). Additionally, I recently ordered all of the behavioral equipment and animal housing required for this project (well over $26,500). Both the Neuroscience Program and CSBS Deans office will contribute $50 each for lab consumables/unforeseen expenses. Additionally, Clarissa is requesting a small stipend to help lost time at work to travel to a conference. This project is going to consume a lot of time as the animals have to be checked daily and the experiments are labor intensive. I would really like to have her attend a conference as that would increase her application for graduate and it will be difficult without this stipend. I believe this budget is appropriate for the size of project and time required from Clarissa.

6. Describe your role in the project.
I will train, mentor and supervise Clarissa on this project. I will provide the entire animal handling training, and we will continue to discuss the project, methods, etc. She will be responsible for first drafts of all data analysis and writings (manuscripts, abstracts, or conference presentations). I will provide edits and help her understand any changes that are made. I have already started to train Clarissa on data entry and analysis using GraphPad prism (using data from past projects). This will help a lot with analyzing results and independence in the project.

7. Include anything else that you think will be helpful to the committee in evaluating this application.
I have experience mentoring undergraduate student and feel confident Clarissa will thrive in the animal lab and with this project. I strongly believe this is a great start to provide the behavioral neuroscience lab. I believe that this research project and OUR funding will significantly enhance Clarissa's graduate school application for Neuroscience programs. This project already has been approved by the IACUC committee (see Appendix C). I am very excited about this project and plan to use the results from this project for an NIH AREA 15 grant submission. The Behavioral Neuroscience Lab has seven student researchers and Clarissa is a lead/senior researcher that is responsible for training new lab members. She is doing a great job and I hope she has the opportunity to have this project funded. Lastly, she won the Big Brain award that is awarded by the Neuroscience Program based on involvement in neuroscience research, activity, and GPA. The Brain Awards are competitive awards.

This project __X__ DOES ____ DOES NOT require review by the WSU Institutional Review Board for Human Subjects or the WSU Animal Care and Use Committee.

Project Mentor Signature

Date 1/15/19

1202
Campus Mail Code

6315
Phone Extension

Cullen KA, Ambrose BK, Gentzke AS, Apelberg BJ, Jamal A, King BA. Notes from the Field: Use of Electronic Cigarettes and Any Tobacco Product Among Middle and High School Students — United States, 2011–2018. MMWR Morb Mortal Wkly Rep 2018;67:1276–1277. DOI: http://dx.doi.org/10.15585/mmwr.mm6745a5.

Hu SS, Neff L, Agaku IT, et al. Tobacco Product Use Among Adults — United States, 2013–2014. MMWR Morb Mortal Wkly Rep 2016;65:685–691. DOI: http://dx.doi.org/10.15585/mmwr.mm6527a1.

Lefever, Timothy W., et al. "Delivery of Nicotine Aerosol to Mice via a Modified Electronic Cigarette Device." *Drug and Alcohol Dependence*, vol. 172, 18 Jan. 2017, pp. 80–87., doi:10.1016/j.drugalcdep.2016.12.004.

Ponzoni, L, et al. "Different Physiological and Behavioural Effects of e-Cigarette Vapour and Cigarette Smoke in Mice." *European Neuropsychopharmacology*, vol. 25, no. 10, Oct. 2015, pp. 1775–1786., doi:10.1016/j.euroneuro.2015.06.010.

Syamlal G, Jamal A, King BA, Mazurek JM. Electronic Cigarette Use Among Working Adults — United States, 2014. MMWR Morb Mortal Wkly Rep 2016;65:557–561. DOI: http://dx.doi.org/10.15585/mmwr.mm6522a1.

Mouse Cost

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Fax: 1-800-992-7329
www.criver.com

Page 1 of 2
Quotation 20189595
04-FEB-2019
10:43 EST
Sold To Party - 101508
Weber State University

Bill To Party - 830212
Weber State University
1202
1299 Edvalson Street
Ogden UT 84408-5137

Ship To Party - 241310
Weber State University
1299 Edvalson Street
Ogden UT 84408-5137

Order Processed by : Mary Eagleston

Information

DUNS	01-971-6729	**EID**	76-0509980
Order No.	20189595	**Terms of Payment**	Net 30 days
Order Date	04-FEB-2019	**Terms of Delivery**	PREPAID
Currency	USD	**Forwarding Agent**	
Purchase Order No.		**Purchase Order Date**	
Scheduled Ship Date			
Valid From	04-FEB-2019	**Valid To**	31-DEC-2019

Comments:

Item	Material	Description	Quantity	Unit Price	Value
10	027C57BL/6	Male C57BL/6 MOUSE 35-35* Days MOUSE - Cohort Order	36 EA	30.30	1,090.80
		Freight			44.70
		Crates			20.65
	Scheduled Ship Date: 08-FEB-2019 Crate Quantity: 1		**Arrival Date: 12-FEB-2019**		
20	027C57BL/6	Female C57BL/6 MOUSE 35-35* Days MOUSE - Cohort Order	36 EA	31.50	1,134.00
		Freight			44.70
		Crates			20.65
	Scheduled Ship Date: 08-FEB-2019 Crate Quantity: 1		**Arrival Date: 12-FEB-2019**		

Sales Tax 0.00

Total Amount
$2,355.50

Food and Bedding Cost

Price Quote

ENVIGO

Date: 2/4/2019
Expires: 3/6/2019
Organization: WEBER STATE
Contact: TODD HILLHOUSE

Price Quote Number: JMC 020419 WSU

Product Code		Price/Unit	Quantity	Total Price
T.2018.15	TEKLAD GLOBAL 18% PROTEIN RODENT DIET	25.20	1	**25.20**
T.7092.40	CORN COB 1/8" BEDDING	20.35	1	**20.35**

Requested Services and Fees

FRT	Freight - Commercial (Estimate)	83.91	1	**83.91**

Refer to product datasheets for key planning information

Total (USD) $129.46

Prepared By:
Jelena Cvetkovich
Jelena.Cvetkovich@envigo.com

Envigo
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Madison, WI 53713
envigo.com

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Payment terms are Net 30 Days

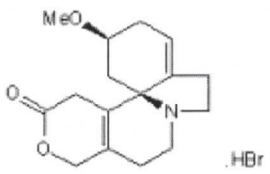
Dihydro-β-erythroidine hydrobromide

Cat. No. 2349 23 Citations Submit a Review

Datasheet / COA / SDS

Pricing	Availability	Qty
10 mg / $195	In Stock	0
50 mg / $815	In Stock	0

Print Quote Bulk Orders Add to Cart



Description: $\alpha_4\beta_2$, muscle type and *Torpedo* nAChR antagonist
Alternative Names: DHβE
Chemical Name: (2*S*,13b*S*)-2-Methoxy-2,3,5,6,8,9,10,13-octahydro-1*H*,12*H*-benzo[*i*]pyrano[3,4-*g*]indolizin-12-one hydrobromide
Purity: ≥98% (HPLC)

Literature

Biological Activity | Technical Data | Solubility | Calculators | Datasheets | References

Biological Activity

Competitive nicotinic acetylcholine receptor antagonist with moderate selectivity for the neuronal α4 receptor subunit (IC_{50} values are 0.19 and 0.37 μM for α4β4 and α4β2 receptors respectively). Antagonizes behavioral effects of nicotine *in vivo*.

Technical Data

M. Wt	356.26
Formula	$C_{16}H_{21}NO_3$.HBr
Storage	Desiccate at RT
Purity	≥98% (HPLC)
CAS Number	29734-68-7
PubChem ID	11957537
InChI Key	GFIGWAJEIMHJJB-LINSIKMZSA-N
Smiles	O=C1CC2=C(CCN4[C@]32C(CC4)=CC[C@H](OC)C3)CO1.Br

The technical data provided above is for guidance only. For batch specific data refer to the Certificate of Analysis.

Tocris products are intended for laboratory research use only, unless stated otherwise.

Solubility Data

	Solvent	Max Conc. mg/mL	Max Conc. mM
Solubility	water	35.63	100
	DMSO	8.91	25

Preparing Stock Solutions

The following data is based on the product molecular weight 356.26. Batch specific molecular weights may vary from batch to batch due to solvent of hydration, which will affect the solvent volumes required to prepare stock solutions.

Concentration / Solvent Volume / Mass	1 mg	5 mg	10 mg

1 mM	2.81 mL	14.03 mL	28.07 mL
5 mM	0.56 mL	2.81 mL	5.61 mL
10 mM	0.28 mL	1.4 mL	2.81 mL
50 mM	0.06 mL	0.28 mL	0.56 mL

Molarity Calculator | Reconstitution Calculator | Dilution Calculator

Product Datasheets

Certificate of Analysis / Product Datasheet

Select another batch: 11 View Batch

Safety Datasheet

References

References are publications that support the biological activity of the product.

Williams and Robinson (1984) Binding of the nicotinic cholinergic antagonist, dihydro-β-erythroidine, to rat brain tissue. J.Neurosci. ***4*** 2906 PMID: 6502210

Damaj *et al* (1995) In vivo pharmacological effects of dihydro-β-erythroidine, a nicotinic antagonist, in mice. Psychopharmacology ***117*** 67 PMID: 7724704

Harvey *et al* (1996) Multiple determinants of dihydro-β-erythroidine sensitivity on rat neuronal nicotinic receptor α subunits. J.Neurochem. ***67*** 1953 PMID: 8863500

If you know of a relevant reference for Dihydro-β-erythroidine hydrobromide, please let us know.

View Related Products by Target

Acetylcholine Nicotinic Receptors | Nicotinic (α4β2) Receptors | Nicotinic Receptors (Other Subtypes)

View Related Products by Product Action

View all Nicotinic Receptor (Other Subtype) Antagonists

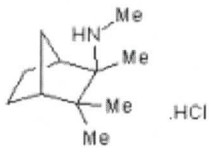
Mecamylamine hydrochloride

Cat. No. 2843 17 Citations Submit a Review

Datasheet / COA / SDS

Pricing	Availability	Qty
10 mg / $95	In Stock	0
50 mg / $399	In Stock	0

Print Quote Bulk Orders Add to Cart



Description: Non-competitive nAChR antagonist
Chemical Name: *N*,2,3,3-Tetramethylbicyclo[2.2.1]heptan-2-amine hydrochloride

Datasheet
Citations (17)
Reviews
Literature

Biological Activity

Non-competitive nicotinic acetylcholine receptor antagonist. Displays antidepressant-like effects in mice.

Technical Data

M. Wt	203.75
Formula	$C_{11}H_{21}N.HCl$
Storage	Desiccate at RT
CAS Number	826-39-1
PubChem ID	13221
InChI Key	PKVZBNCYEICAQP-UHFFFAOYSA-N
Smiles	CC1(C)C2CCC(C2)C(NC)1C.Cl

The technical data provided above is for guidance only. For batch specific data refer to the Certificate of Analysis.

Tocris products are intended for laboratory research use only, unless stated otherwise.

Solubility Data

	Solvent	Max Conc. mg/mL	Max Conc. mM
Solubility	water	20.37	100
	DMSO	15.28	75

Preparing Stock Solutions

The following data is based on the product molecular weight 203.75. Batch specific molecular weights may vary from batch to batch due to solvent of hydration, which will affect the solvent volumes required to prepare stock solutions.

Concentration / Solvent Volume / Mass	1 mg	5 mg	10 mg
1 mM	4.91 mL	24.54 mL	49.08 mL
5 mM	0.98 mL	4.91 mL	9.82 mL
10 mM	0.49 mL	2.45 mL	4.91 mL

50 mM	0.1 mL	0.49 mL	0.98 mL

Molarity Calculator | Reconstitution Calculator | Dilution Calculator

Product Datasheets

Certificate of Analysis / Product Datasheet

Select another batch: 3 **View Batch**

Safety Datasheet

References

References are publications that support the biological activity of the product.

Ostroumov *et al* (2007) Modeling study of mecamylamine block of muscle type acetylcholine receptors. Eur.Biophys.J. ***37*** 393 PMID: 17938901

Rabenstein *et al* (2006) The nicotinic antagonist mecamylamine has antidepressant-like effects in wild-type but not β2- or α7-nicotinic acetylcholine receptor subunit knockout mice. Psychopharmacol. ***189*** 395

If you know of a relevant reference for Mecamylamine hydrochloride, please let us know.

View Related Products by Target

Acetylcholine Nicotinic Receptors | **Nicotinic Receptors (Non-selective)**

View Related Products by Product Action

View all Nicotinic Receptor (Non-selective) Antagonists

Keywords: Mecamylamine hydrochloride, Mecamylamine hydrochloride supplier, Non-competitive, nicotinic, receptors, antagonists, Acetylcholine, Non-Selective, Subtypes, nAChR, Nicotinic, Receptors, (Non-selective), 2843, Tocris Bioscience

Contact Information

Tocris is a Bio-Techne brand. To place orders, for customer services and technical support please contact:

SIGMA-ALDRICH

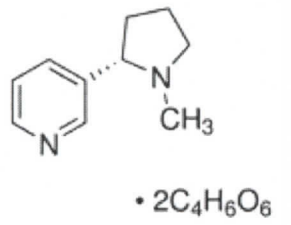
SML1236 Sigma-Aldrich

(−)-Nicotine hydrogen tartrate salt

≥98% (HPLC)

Synonym: (−)-1-Methyl-2-(3-pyridyl)pyrrolidine (+)-bitartrate salt, (−)-Nicotine ditartrate

CAS Number 65-31-6 | **Empirical Formula (Hill Notation)** $C_{10}H_{14}N_2 \cdot 2C_4H_6O_6$ | **Molecular Weight** 462.41 | **EC Number** 200-607-2 | **PubChem Substance ID** 329825736



$\cdot 2C_4H_6O_6$

SDS

Similar Products

SKU-Pack Size	Availability	Price (USD)	Quantity
SML1236-50MG	Available to ship on 02/04/19 - FROM	64.40	0
SML1236-1G	Available to ship on 02/04/19 - FROM	1,050.00	0

Bulk orders?

ADD TO CART

Properties

Related Categories	Agonists, Bioactive Small Molecule Alphabetical Index, Bioactive Small Molecules, Cell Biology, Cell Signaling and Neuroscience, More...
InChI Key	RYYKFQGHMYGLEL-PPHPATTJSA-N
assay	≥98% (HPLC)
form	powder
optical activity	[α]/D +20 to +26°, c = 1.0 in water
storage condition	desiccated
color	white to beige
solubility	H_2O: 20 mg/mL, clear

Show More (9)

Description

Biochem/physiol Actions
Prototype nicotinic acetylcholine receptor agonist; naturally occurring isomer.

Nicotine hydrogen tartrate (NHT) is a biodegradable polymer of chitosan. NHT is considered to be more stable than nicotine.[2] Nicotine is highly addictive drug and is indirectly but strongly associated with tobacco related diseases. It helps to discontinue smoking. Nicotine might serve as a therapeutic agent in treating Alzheimer's disease, Parkinson's disease and ulcerative colitis.[3]

Packaging
1 g in glass bottle

50 mg in glass bottle

Application
(−)-Nicotine hydrogen tartrate salt has been used as an agonist for acetylcholine receptor (AChr).[1]

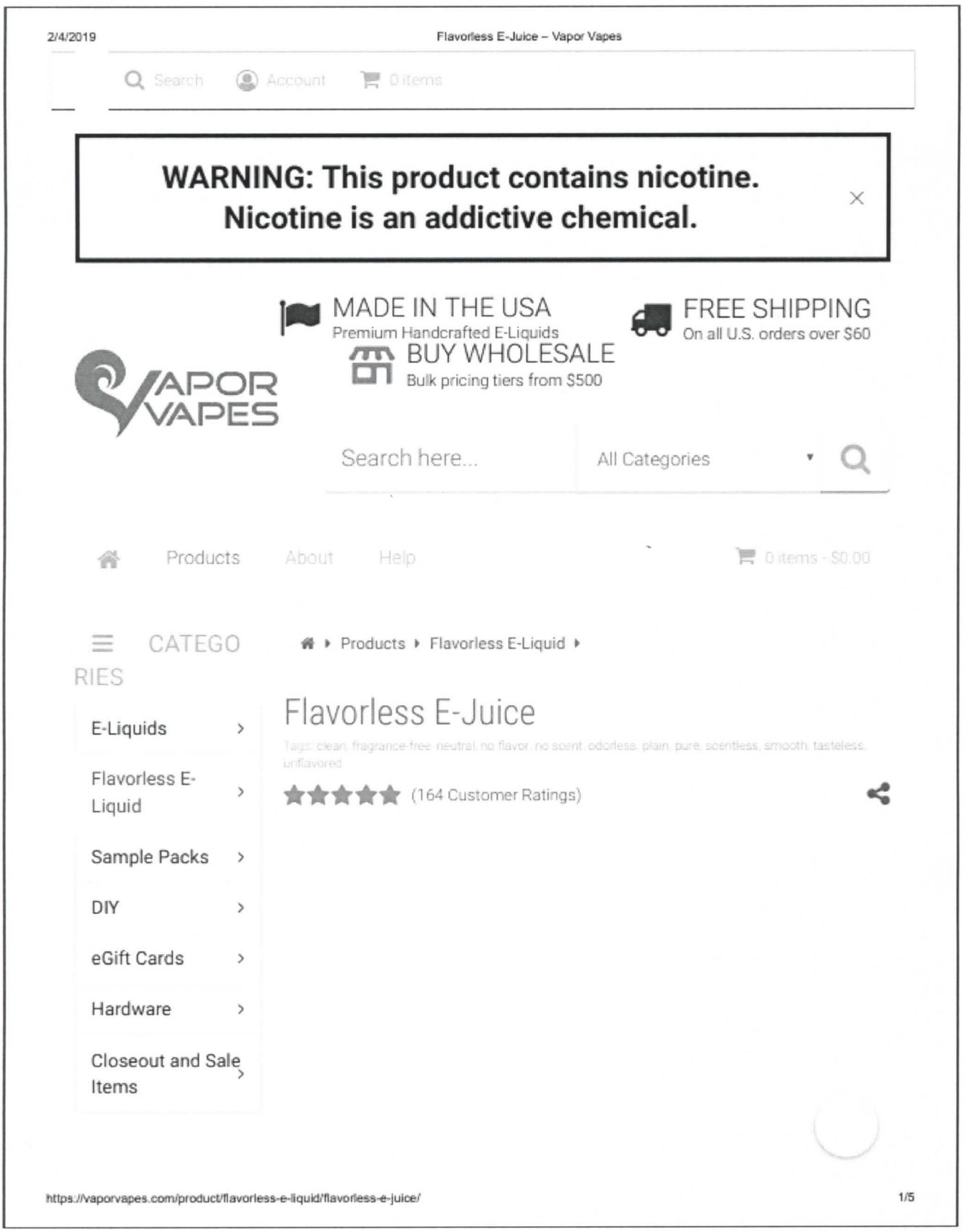
(−)-Nicotine hydrogen tartrate salt has been used in intracellular calcium imaging.[1]

Safety Information

RIDADR UN 1659 6.1 / PGII

Documents

Certificate of Analysis Certificate of Origin


2/4/2019
Flavorless E-Juice – Vapor Vapes
Search
Account
0 items
WARNING: This product contains nicotine.
Nicotine is an addictive chemical.
MADE IN THE USA
Premium Handcrafted E-Liquids
FREE SHIPPING
On all U.S. orders over $60
BUY WHOLESALE
Bulk pricing tiers from $500
VAPOR VAPES
Search here...
All Categories
Products
About
Help
0 items - $0.00
CATEGORIES
E-Liquids
Flavorless E-Liquid
Sample Packs
DIY
eGift Cards
Hardware
Closeout and Sale Items
Products ▸ Flavorless E-Liquid ▸
Flavorless E-Juice
Tags: clean, fragrance-free, neutral, no flavor, no scent, odorless, plain, pure, scentless, smooth, tasteless, unflavored
(164 Customer Ratings)
https://vaporvapes.com/product/flavorless-e-liquid/flavorless-e-juice/
1/5

Search Account 0 items

- Flavorless E-Juice from $8.99 (164)
- Travel Bottle from $0.70 (3)
- Coffee Bean Tob... from $2.50 (15)
- PG/VG Base from $8.99 (3)
- Black Currant E-J... from $2.50 (6)
- Wintergreen E-Ju... from $2.50 (8)
- Sample 5-Pack (... $24.99 (0)
- Sample 5-Pack (... $9.99 (2)
- Cherry Slush E-J... from $2.50 (7)
- Mango E-Juice from $2.50 (15)

Bottle Size:	1000ml / 33.8oz
Bottle Type:	Plastic Bottle
Nicotine:	0mg - 0% No Nicotine
Mix:	PG/VG 50/50 Best Mix

Price: **$43.99** ✔ In Stock

− 1 + Add to Cart

Schedule your order and **SAVE 10%**

Description Reviews (164)

This unflavored e-juice is a Vapor Vapes favorite. The pure blend USP grade Kosher Propylene Glycol (PG) and Vegetable Glycerin (VG) provides a smooth, clean and premium vaping experience you can enjoy on its own.

Weber State University
IACUC Laboratory Animal Protocol

1. Name of Principal Investigator: Todd Hillhouse

Title: Assistant Professor

Dept./Phone: Psychology/ (801)626-6315

Ia. Name of Co-investigator(s):

Title:

Dept./Phone:

2. Type of Project:

[X] Research

[ ] Class (name/number):

[ ] Other

3. Project Title:
Evaluating the rewarding properties of Electronic-cigarettes (E-cigs) using self-administration in mice

4. Period Covered by this Proposal:

Starting Date: 4/12/2018

Completion Date: 41121.2020—

5. Funding Source: Hillhouse Start-up Funds and OUR research grant awarded to Todd Sarah oneycutt

6. Species and the Approximate Total Number of animals for project: Species
   1. C57BL/6 mice -60 600 (see amended procedures)
   2. _____ _____
   3. _____ _____

7. Check the appropriate items. Please include explanation for all checked items in narrative under Section II.

   [ ]Field Study
   [ ]Captive observations
   [X]Behavioral conditioning is required
   Animals collected from wild
   [ ]Tissue biopsy (including blood)
   [X]Drug administration
   [ ]Antibody production (or other immunological technique) is required
   [ ]Biological or radiological hazardous agents are required
   [ ]Tumor production required
   [ ]Long-term restraint is required
   [ ]Survival surgery is required [ ]Non-survival surgery is required [ ]Other (please explain):

8a. Where will the animals be obtained?
All mice will be obtained from commercial research breeders such as Envigo, Charles River Laboratories, Hilltop Lab animals, and Jackson Laboratory. Additionally, I pay to purchase breeding pair to breed my own animals that can be used in this study. A breeding protocol will be submitted as a separate IACUC protocol.

8b. Will the animals be housed? [ ] No. [X] Yes. Where2-SL6.6A— Please explain the husbandry protocol below or attach:
All mice will be housed in accordance with Guide for the Care and use ofLaboratory

Animals 8th edition. Mice will be group housed (n =4 per cage) in standard "shoe box cages" (7.5 Inces WI corn co e mg, ree access ro en c ow an wa er, aco square for enrichment. The cage some equipped with a stainless steel bar lid, plastic filter top and filter. These were purchased from Allentown. Animals will be maintained on a 12 hour light/dark cycle with lights on at 7am. The room will be maintained between 20-26^{0}C.

9. Disposition of animals upon completion of project:

Mouse carcass's will be stored in a chest freezer marked as bio-hazard waste (only carcass' will be stored in the freezer). When needed the carcass' will be dispose through a vet office. Euthanasia will be by C02 asphyxiation (SOP is attached to protocol). Animals that have received placebo or no treatment may be given to the Zoology snake lab as animal feed.

10. State/Federal approval (if appropriate):

Il. As required by 9 CFR § 2.31(d)(1)(i-xi) and 9 CFR § 2.31(e)(I-5), please attach a narrative description of the proposed study in response to the prompts listed below. Write the narrative in sufficient detail and responsiveness to the prompts so that it can be appreciated by a well-informed lay-person such that said lay-person can determine whether adequate measures are in place to ensure the humane treatment of animals and the compliance with federal law.

a. Discuss the scientific merit of the proposal by referring to relevant research to justify the use of animals, and/or discuss the lack of research conducted on the research question and how a study on said research question would provide significant results sufficient to justify the use of animals. More specifically include the rationale for the selection of the species, gender (if appropriate) and number of animals, and justify said rationale with references to relevant literature and statistical modeling. In this write up, investigators must provide assurances that the minimal number of animals required will be used.

b. Provide a complete description of all procedures and manipulations to be used in your project that involve animals. All items checked in section 7 of the protocol must be sufficiently described so that a lay person can reasonably appreciate the procedures and manipulations.

c. Discuss how the procedures will minimize the discomfort, distress and pain to animals

d. (Where applicable) Discuss how you have considered alternatives to procedures that may cause more than momentary or slight pain or distress to the animals with a written narrative of the methods and sources.

e. Discuss and provide assurances about how this procedure does not unnecessarily duplicate previous experiments.

f. (Where applicable) Discuss the following when procedures cause more than momentary or slight pain or distress to the animals:

i. How the procedure will be performed with appropriate sedatives, analgesics or anesthetics or the scientific reasons for withholding said pain relievers; ii. How the procedures will not extend beyond the time necessary for the study; iii. How you plan on consulting with the attending veterinarian or his/her designee; iv. If paralytics are used, how anesthesia will be implemented.

g. (Where applicable) If the animals will experience severe or chronic pain or distress which cannot be relieved, first justify why euthanasia is appropriate. Second discuss how euthanasia will be performed including the agent, methods and timing. Third discuss how the euthanasia qualifies as either a method that produces rapid unconsciousness and subsequent death without evidence of pain or distress, or alternatively a method that utilizes anesthesia produced by an agent that causes painless loss of consciousness and subsequent death. If the euthanasia techniques deviate from the two described methods above, explicitly state the difference in the techniques and provide scientific justifications for the deviations citing relevant sources.

h. Discuss how the animals' living conditions will be appropriate for their species and specifically refer to relevant passages of code in 9 CFR § 3 (see http://www.law.cornell.edu/cfr/text/9/part-3) to justify how the animals' living conditions are at least compliant with federal regulations and where provided compliant with the guidance of the attending veterinarian and the policies and procedures of Weber State.

i. Discuss how the animals will receive medical care when necessary from a qualified veterinarian.

j. Discuss how the personnel conducting the animal manipulations/procedures are and/or will be appropriately qualified and trained to perform those procedures. Include any animal care and training program that the personnel have completed or document the amount of experience that personnel have had conducting the procedures

12. Principal Investigators Warranties and Representations:

a) I agree and acknowledge that IACUC is authorized by federal law to approve or withhold approval of protocols and require revisions of said protocols, and as such, I shall be compliant and work with IACUC to ensure the humane treatment of animals used as research subjects. I further agree and acknowledge that I shall not circumvent the IACUC process when conducting research using animal subjects in this protocol or future protocols.

b) If the procedures, etc., herein require revision (change in procedure, species, numbers, etc.), I will make two written requests for authorization to the chair of IACUC. It is the responsibility of the investigator to submit a memo each year to indicate if the project will be done that year and to indicate any changes.

c) I hereby warrant and represent that I shall conform to all information contained in this protocol and follow the regulations set forth by the USDA under the provisions of the Animal Welfare Act and the Policy on Humane Care and Use of Laboratory Animals as established by the Public Health Service and that the information provided above is accurate to the best of my knowledge.

12/6/18

Principal Investigator Date

Co-Investigator Date

11-16-18

IACUC Chair Date

11-30-18

Veterinarian Date Co-Investigator Date

Section B (If Applicable)

A) Surgical Procedures:

1. Indicate the following I) where the surgery will be performed and whether the facility is intended for that purpose, 2) the person(s) performing the surgery, and 3) the qualifications and experience of the person(s) to perform the techniques involved (aseptic techniques must be used during surgery including surgical gloves, masks, and sterile instruments).

2. Describe the surgery procedure including all support care and monitoring during the procedure.

3. Anesthesia,

Species	Anesthetic	Dose	Route	Duration

4. Please describe method of measuring/monitoring depth of anesthesia.

5. Describe any pre-operative procedures, including fasting (food, water) and preanesthetics.

6. If survival surgery, describe postoperative survival time, care (and who will give it), including:

Recovery:

Supportive care:

Postoperative monitoring:

Analgesia:

Antibiotics:

After hour care, etc.:

B) Immunization/Antibody Production:

Species	Agent	Route	Site	Volume	# of Doses	Interval

6

C) Hazardous Agents:

Agent(s):

Biosafety & Controlled Substance Approval:
(Richard Sandau-MC 3002; x8004) [J Yes No [Pending [ ] N/A Radiation Safety Committee Approval:
(MC2508•, x7982) [J Yes [J No [ ] Pending [ ] N/A

7. Describe the method, route, place of administration, personnel involved, precautions to protect people and animals, and carcass disposal.

IIa. Background, scientific merit, and information about animals

While the number Americans that smoke traditional cigarettes has decreased approximately 5% over the last decade, electronic-cigarettes (E-cigs) and electronic nicotine delivery systems (ENDs) use has increased to approximately 10% of the United State population (Hu et al, 2016; Jamal et al, 2016; Hillhouse et al., preliminary data). According to the Food and Drug Administration (FDA), over a four-year period (2011-2015) ENDS use among middle school and high school students rose from 1.5% to 16% with more than 2 million students identifying as current ENDS users in 2016 (FDA, 2018). ENDS are products that include a power source (a battery), a heating element (commonly referred to as an atomizer) that vaporizes the tobacco-less nicotine solution (called E-juice or E-liquid), and a compartment (called a "tank") to hold the E-liquid solution. E-liquid solutions can varying in nicotine concentrations (0-42 mg/ml.) (Etter, 2012), There are street names for ENDS (E-cigs, Vape pens, Mods, Vapes, etc.) are based on the size and design of the device. For this proposal, we will use the term ENDS because it is a term that includes all the devices that are currently on the market. When the consumer inhales from ENDS, the E-liquid is vaporized by the atomizer and the nicotine is administered to the consumer through the lungs like a traditional cigarette. Ultimately, ENDS manufacturers claim that these devices are a "safer" option compared to traditional cigarettes. These claims are based on the idea that E-liquid contains fewer carcinogens than traditional cigarettes because it is not using combustion as the mechanism for delivering the vapor (or smoke). Clinical research has shown that there is no carbon monoxide (CO) exposure after ENDS use (Spindle et al., 2015). Additionally, ENDS have been marketed as a smoking cessation device for current cigarette smokers. However, the scientific community is still investigating the safety and health risks associated with long-term ENDS use. While Ecigs and ENDS are generally considered as a safer alternative to traditional cigarettes, the

overall safety and health risks associated with these devices are not fully understood. Several studies have found cancer causing (e.g. formaldehyde, acetaldehyde acetone) and endocrinedisrupting (e.g.flame retardants) chemicals in the E-liquid and urine of ENDS users, respectively. Nicotine is classified as apsychostimulant and is considered a drug of abuse. This significant increase in drug taking behavior, especially among young teens, may lead to nicotine addiction, dependence, and long-term use. The first AIM of the proposed research is to evaluate the rewarding properties of ENDS, which are directly related to drug taking behavior. We will be using a 'E-vape" mouse self-administration chamber to evaluate the rewarding properties of ENDS in mice. Self-administration procedures in the laboratory allow for drugtaking behaviors similar to the real world. We hypothesis that two mechanisms will be responsible for the rewarding properties of ENDS: I) nicotine and 2) appealing flavors.

The proposed species, strains, and number were chosen based on previous research in the area. While rats are typically use for self-administration because it is easier to place an IV catheter, our study does not require surgery and thus we have chose to use mice for this study. Our studies will use male C57BL/6 mice which are one of the most commonly used in-bred mouse grain available, which allows is to make direct comparisons to previous published literature. Additionally, this mouse (C57BL/6) is commonly used at the background strain for many genetically altered mice (specifically gene knockout mice), which will allow us to make comparisons to genetically altered mice in future studies. Mice will between the ages of 830 weeks at the time of experimental testing. The variability inherent in the self-administration requires 8 to 12 animals to be tested with each drug dose in order to reveal statistically significant effects (power = .80, effect size = 0.25 [eta squared], alpha = 0.05). We will use a within subjects (repeated measures) research design to minimize number of mice used in the gudy. Our research design requires testing one group ofmice (N = 8-12) to establish a dose response curve with standard E-liquid purchased from a vape shop,

two group of mice (N = 8-12 each group) to complete antagonist (two antagonists), and two group of mice (N = 8-12 each group) to complete the E-liquid flavor However, we will use mice in multiple experiments, ifpossible, to reduce the number ofmice needed. The maximum number ofmice required for the study are as follows: Dose response curve (N = 12) mice; Antagonist study N — 24; Flavor study N = 24. Maximal total mice require for to complete the study is 60. However, we will tests the minimum number of doses and mice (at each dose) to reduce the total number of mice required to evaluate our research question. Nicotinic antagonist were administered by subcutaneous (sc.) or intraperitoneal Op.) injection at a volume of 10 mL/kg.

This is an amendment for my "Evaluating the rewarding properties of Electronic-cigarettes (E-cigs) using self-administration in mice" protocol (#184). The additional studies added to this protocol will allow use to fully understand that behavioral effects of E-cigs, which have not been reported in the literature. Specifically we want of this study will be to I) Evaluate the abuse-related effects using a condition placed preference (CPP) design, 2) evaluate the effects on locomotor activity using an open field test, 3) evaluate the antidepressant-like effects in the forced swim test (FST), 3) evaluate anxiolytic-like effects using the noveltyinduced-hypophagia (NIH). The following behavioral procedures have been approved in a different IACUC protocol (18-02): Locomotor activity, FST, and NIH.

The proposed species, strains, and number were chosen based on previous research in the area. The CPP, locomotor activity, FST, and NIH are most commonly used in mice. Our studies will use male C57BL/6 mice which are one of the most commonly used in-bred mouse strain available, which allows is to make direct comparisons to previous published strain for many genetically altered mice (specifically gene knockout mice), which will allow us to make comparisons to genetically altered mice in future studies. Mice will between the ages of 8-30 weeks at the time of experimental testing. The variability inherent in the CPP, FST, NIH, and open field procedures requires 8 to 12 animals to be tested with each drug dose in order to reveal

statistically significant effects (power = .80, effect size = 0.25 leta squared], alpha = 0.05). We will use a between subjects research design to minimize distress for each mouse for FST, NIH, and CPP. Locomotor activity will use a within subject design. Our research design for FST and NIH requires testing 4-6 doses including the placebo control of Vape juice, a nicotine injection as a positive control (4 doses including the placebo control of (-)nicotine and an antidepressant /anxiolytic positive control fluoxetine, imipramine, bupropion or other antidepressant and anxiolytic drugs (4 doses including the placebo control) for each of the procedures. The maximum number of mice required for the FST, NIH, are as follows: Vape administration (N = 8-12 per dose) x 6 doses (0-32.0 mg/ml) = 72 mice; (-)nicotine injection (N = 8-12 per dose) x 4 doses (0-3.2 mg/kg) = 48 mice; Antidepressant drugs (N = 8-12 per dose) x 4 doses (0-32.0 mg/kg) = 48 mice. A maximum total mice required for each procedure is 168 (Total for FST and NIH behavioral assays = 336). The maximal total mice require for to complete the Locomotor assay are as follows: Vape administration (N = 24) will use a repeated measure design; (-)nicotine injection (N = 24) will use a repeated measure design. A maximum total mice required for the locomotor activity study is 48 mice. The maximal total mice require for to complete the CPP study are as follows: Vape administration (N = 8-12 per dose) x 6 doses (0-32.0 mg/ml) = 72 mice; (-)nicotine injection (N = 8-12 per dose) x 4 doses (0-3.2 mg/kg) = 48 mice. A maximum total mice required for the CPP test is 120. The proposed amendment will require a maximum 504 additional mice. However, we will tests the minimum number of doses and mice (at each dose) to reduce the total number of mice required to evaluate our research question. volume of 10 mL/kg.

Additionally, we would like to increase the number of mice for the self-administration from 12 to 36. Training this procedure has been more difficult than thought because there are no published methods for this procedure. We have used 12 mice thus far trying o train mice to self-administer. We need additionally mice to establish the procedure and conduct the dose response curve.

11b. description of all procedures

E-cig SelfAdministration in Mice

Dose Response Curve: To conduct self-administration of E-cigs we will be using an E-vape apparatus. This equipment is cutting-edge and only a few laboratories in the United States are using E-vape chambers for research in rodents. The mini-computer allows for control of the number of response required for vapor administration, duration of the puff, number of puffs, turns on/off the cue lights, duration of session, etc. The airtight chamber made of clear plastic and features a HEPA filter for vapor exhaust, which will allow us to use the chamber in the animal facility without risk of exposure to the researcher (see product fact sheet in appendix). We have modified self-administration procedures to align with the equipment and delivery system of E-vape (Jones & Comer, 2013). There will be a habituation phase, training phase, and test phase. During the habituation phase mice will be placed in the chamber and be exposed to passive (no response require) puffs of E-cig vapor (0-30 mg/mL). During these sessions will receive several puffs over a 30 min period of time and with each puff a cue light will flash. Cue lights are located over the nose poke response. After several days of the habituation phase, mice will move on the training phase. During the training phase mice will be require to make increase number of responses to receive a puff of vapor. After behavior have stabilized and mice consistently self-administer vapor we will start the dose response testing phase. During this phase, mice will self administer various concentrations ofvape (0-30 mg/ml). This will allow us to determine how steep the dose response curve is for E-liquid and determine if any doses are considered aversive. We will purchase nicotine free E-liquid from an online vape store. We will nicotine powder from Sigma Aldrich to make our own nicotine concentrations.

Antagonist Studies. The antagonists and methods chosen for this study were adapted from (Freita et al., 2015; Freita et al 2016; Jones & Comer, 2013). Antagonist studies will be conducted identical to

the dose response studies. For the antagonist students, mice will receive a pretreatment (30-60 mins) of the nonselective nicotinic acetylcholine receptor (nAChR) antagonist Mecamylamine and the selective a4p2 antagonist dihyrdo-P-erythroidine (DHPE) and various concentrations of nicotine E-liquid (doses will be based on the first dose response curve). This will allow us to determine if the reinforce properties of E-liquid is a nonspecific effect on nAChR or a4p2 specific.

Flavor Study. The flavors and methods chosen for this study were adapted from (Freita et al., 2015; Freita et al 2016; Jones & Comer, 2013). Flavor studies will be conducted identical to the dose response studies. Inthis study, the mice will be given different flavors of E-liquid at various nicotine concentrations (doses will be based on the first dose response curve) to determine if the flavor of E-liquid can increase or decrease the rewarding properties of E-cigs.

Conditioned Place Preference (CPP): Mice were trained in a standard two chamber conditioned placed preference (CPP) box (Med Associates Inc.). The CPP box consisted of a white side and black side that were separated by a neutral wall equipped with a guillotine style door. The white side was equipped with a mesh floor; whereas, the black side was equipped with a steel bar floor. Each side was equipped with a small light on the Plexiglas top that provided consist illumination in both chambers. These chambers has IR photobeam detectors to access locomotor activity.

Acquisition of Conditioned Place Preference.CPP training were adapted from published (Brabant et al., 2005; Szumlinski et al., 2002; Carey et al., 2007; McLaughlin et al., 2003; Ignatowska-Jankowska et al., 2013) and preliminary experiments while at University Of Michigan. CPP experiments consisted of five phases: Bias test (day I), conditioning training (can be up to 10 days) preference test day, and extinction training (day 5+ until criteria was met). A bias test session will be conducted on day one in which mice were placed in the box and allowed to freely explore both sides of the CPP box for 30 min. Time spent in each side will be recorded. An unbiased counterbalanced design to assign the drug paired side. For example, approximately half the mice will be assigned nicotine on the side in which they had a preference and the other half will be assigned

saline/vehicle on the side in which they had a preference. Mice that had a strong preference for one side (>70% of time spent on one side) will be eliminated from the study after the bias test session. Training consist of several days of saline and nicotine drug pairings. On training days, mice received saline/vehicle

(vapor or injection) in the morning and will be confined to the saline-paired side for 30 min. In the afternoon mice received (-)nicotine (vapor or injection) and will confined to the drugpaired side for 30 min. The preference test session was conducted after all training sessions are complete. No vapor or injections will be given prior to the preference test session and mice were free explore both sides of the CPP box for 30 min. Time spent in each side will be recorded for the test session.

Extinction Training. Next, mice will receive daily extinction training (Monday-Friday). For extinction sessions, mice will not receive vapor or injections but mice were free to explore both sides of the CPP box for 30 min. Time spent in each side will be recorded. Extinction training continued until the preference for the nicotine-paired side is reduced by 50% of the initial test preference. Mice that did not extinguish their preference after 40 extinction sessions were eliminated from the session.

Forced Swim Test (FST): The FST procedure is based on published literature (Li et al., 2010; Maeng et al., 2008; Sunal et al., 1994). The forced swim test (FST) will be used to evaluate the antidepressant-like effects of HA-966 and controls in mice. FST is the most common behavioral screening protocol to assess antidepressant-like effects of novel compounds. Mice will receive an injection of HA-966 (0-32 mg/kg) or MK-801 (0-0.3 mg/kg; glutamate positive control) o r fluoxetine (0-10 mg/kg; antidepressant positive control) 30 min prior to the FST. For the FST, mice will be placed in a glass cylinder (18 cm tall x 14 cm in diameter) filled with water to a depth of 14 cm for 6 mins. Test sessions are 6 min in duration and videotaped. Mice will be continuously monitored during the swim procedure to prevent accidental drowning. Mice are tested individually (i.e. one mouse at a time). A trained blinded observer scored the immobility time (seconds) for each 6 min test session.

Immobility was defined as mice motionless without any escape-related behaviors. Student researchers will be trained by watching the Journal of Visualized Experiments (JOVE) video on FST (Can et al., 2012). To maintain appropriate statistical power, we will need 10 mice per dose: 40 mice for HA-966 dose response curve and 20 mice for MK-801 control.

Novelty-induced Hypophagia (NIH): Test procedures were adapted from published literature (Dulawa et al 2004; Talbot et al 2010). Mice will group-housed (4 per cage) to start NIH training. On days 1 and 2, mice will receive overnight access to water in standard volumetric sipper tubes (Med Associates Inc) as a means to acclimate the mice to the sipper tubes. On days 3 and 4, mice will have access to a sweetened solution (Ensure@ at a water:Ensure ratio of 1:2) for 2-4 hours. After these four days of group-housed training, mice will be single-housed for the remainder of the study. For three consecutive days (days 5-7), training sessions will be conducted in their home cage in which the mice will have 30 min access to the sweetened solution. On day 8, a home cage test session will be conducted in which time to drink and amount consumed will be measured. On day 9, a novel cage test session will occurr in which mice will be placed into a new larger cage that is similar in color, but will not have bedding. The home cage and novel cage test sessions will be 30 min in duration and the latency to drink and volume consumed were recorded. A trained blinded observer scored test sessions.

Open Field Test: Procedures and rational was based on published literature or preliminary data (Engin et al., 2009; Hillhouse et al., Submitted). The open field testing apparatus is a clear Plexiglas box l**0.75×10.75×8** inches. Test sessions will be conducted in three standard open field activity chambers enclosed within sound attenuating cubicles (Med-Associates). Each chamber was equipped with three 16-beam IR arrays, which track the mice throughout the chambers, and a house light. Activity Monitor (version 7; Med-Associates) was used to collect the data provided from the three 16-beam IR arrays. All animals will be placed in the same corner of the open field at the

beginning of the test. Four measures will taken during the 30-60 min test sessions: (1) the number of line crosses (i.e. horizontal activity), (2) the number of rearing behaviors (i.e. vertical activity, defined as raising both forepaws above the floor while balancing on hind limbs), (3) the amount of time spent in the center of the field, as defined by all four paws being in the center 16 segments of the apparatus, and (4) the number of fecal boli left in the apparatus. Vertical and horizontal activities were taken as measures of locomotor activity. activity in the center and number of fecal boli was taken as an inverse measure of anxiety (Engin et al., 2009).

Drug administration: All drugs will be dissolved into sterile water or 0.9% saline solution.

Drugs will be stored according to MSDS and will be kept in a refrigerator after they have been dissolved in the appropriate solution. All drugs were administered by subcutaneous (s.c.) or intraperitoneal (i.p.) injection at a volume of 10mL/kg. Drugs will be administered 0-60 minutes prior to experimental sessions. Mecamylamine is a nonselective nicotinic acetylcholine receptor (nAChR) antagonist. dihyrdo-p-erythroidine (DHPE) is a selective n4P2 antagonist, or other related nicotinic antagonists (Freita et al., 2015;Freita et al 2016). We will purchase nicotine free E-liquid from an online vape store. We will nicotine powder from Sigma Aldrich to make our own nicotine concentrations. I would like to expand the drugs that are available under this protocol to allow flexibility based on the studies results and procedures used. This will allow us to explore the mechanism and compare the results to the most appropriate control which is not listed above. We would like to add antidepressant drugs to serve as controls for some of the new procedure mentioned above (FST, NIH, etc), which include but are not limited to,

Imipramine (tricyclic antidepressant: doses 0-32.0 mg/kg), Bupropion (atypical antidepressant drug - Dopamine/Norepinephrine reuptake inhibitor: doses 0-32.0 mg/kg),

Duloxetine (serotonin-norepinephrine reuptake inhibitor: doses 0-32.0 mg/kg), and Buspirone (serotonin partial agonists: doses 0-32.0 mg/kg). Pharmaceutical grade antidepressants will not be used in this study for two reasons: I) pharmaceutical grade

nnfidønrpqqnntq nnt in a farm which is required for the dose curve. 2) other research uses antidepressants purchased from Sigma Aldrich, which is where we plan to purchase the antidepressants and it will allow for better replication to previous published literature. Additionally, We plan to explore the interaction between the nicotinic (acetylcholine) system and cannabinoid (CB) system as the cannabinoid systems has been implicated in the abuse related potential of nicotine; however, this relationship has yet to be evaluated with vaporized nicotine (Ignatowska-Jankowska et al., 2013). To evaluate this relationship we will use various CBI and CB2 antagonists which include, but are not limited to, SR147778 (CBI antagonist: Doses 0-32.0 mg/kg), SR 141716 (CBI antagonist: 0-32.0 mg/kg), and SR 144528 (Rimonabant, CB2 antagonist; doses 0-32.0).

IIc. Discuss Discomfort, distress and pain

The proposed research are all basic behavioral studies and I have categorized these studies by pain category (attached is a form with the breakdown of the different pain categories).E-vape self-administration is considered pain category C procedure, which are non-invasive, and low impact study that do not involve pain and/or distress. Thus, these gudies do not require the use of anesthetics, analgesics or tranquilizers. This type of self-administration can be considered open-field and/or positive reward conditioning. No surgery is required. The proposed research are all basic behavioral studies and I have categorized these studies by pain category (attached is a form with the breakdown of the different pain categories). The Conditioned place preference, novelty-induce hypophasia and open field test are considered pain category C procedure which are non-invasive and low impact study that do not involve pain and/or distress. Thus these studies do not require the use of anesthetics, analgesics or tranquilizers. The forced swim test is considered Pain

Category E because it causes more than slight or momentary pain or distress. Animals will receive relief from the distress associated with FST by immediate removal from water and towel lid. Alternatives to Forced Swim Test

The forced swim test is the most commonly used behavioral test to evaluate novel antidepressant drugs. This test also has the best predictive validity in terms of which antidepressant will be effective in humans. There are no comparable behavioral test in Pain category C that can be used for this purpose. The other comparable behavioral test is the tail suspension test, which is also pain category E.

Ile. Unnecessary duplication ofpervious experiments.

The E-vape chamber is fairly new and only a few labs in the United States have this chamber. There are no publications on self-administration of E-cigs in mice. There are not published data on the behavioral effects of Vape administered nicotine in mice. Injected nicotine must be used to directly compare administration routes.

llf-g. Pain Category E studies

The forced swim test is considered Pain Category E because it causes more than slight or momentary pain or distress. Animals will receive relief from the distress associated with FST by immediate removal from water and towel drying and heat lamp warmth will be provided.

Uh.Livingconditions

All mice will be housed in accordance with Guidefor the Care and use ofLaboratory Animals gth edition. Mice will be group housed (n =4 per cage) in standard "shoe box cages" (7.5 x 5.0 X 11.75 inces) with com cobb bedding, free access to food and water, a cotton square for

enrichment. The cage some equipped with a stainless steel bar lid, plastic filter top and filter. These were purchased from Allentown. Animals will be maintained on a 12 hour light/dark cycle with lights on at 7am. The room will be maintained between 20-26^{0}C.

Ili. Contacting Veterinarian

For vet care we notify Dr. Aaron Olsen our IACUC vet. I will use the method Phone/EmaiI that is preferred by Dr. Olsen.

llj. Personnel training.

I have worked for 9 years and have over 13 peer review articles in the field of behavioral neuroscience. My graduate work at Northern Michigan University (MS) and Virginia Commonwealth University (PhD) as well as my Postdoctoral Fellowship at University of Michigan focused on evaluating novel drugs at therapeutics for various mental disorders or addiction. I have used all of the proposed experimental procedures in the past. I have completed several animal care and laboratory based trainings over my academic career which include: Animal handling, bloodborne pathogens, Chemical Lab safety, Introduction to mice and rat research, Orientation for animal care and use, and radiation training. I will personally train any student that works in my lab and have them complete CITI training. Everyone will wear Personal Protection Equipment (PPE) which include lab coat, shoe covers, hair bonnet, gloves, and safay glasses (when needed).

Etter, J .F. (2012) Commentary on Wagener etal. (2012): Electronic cigarettes -the Holy Grail of Nicotine Replacement? Addiction, 107, 1550-1552. doi:lO.l111/j.1360-0443.2012.03909.x

Freita KC, Carroll FI, Negus SS (2015) effects of nicotinic drugs on pain-depressed behavior. JPET, 355, 343-352.

Freita KC, Carroll FI, Negus SS (2016) Comparison of effects produced by nicotine and the a4P2-selective agonist 5-14-85380 on intracranial self-stimulation in rats. Exp Clin Psychopharmac 01, 24, 65-75

Hu S. S Neff L. , Agaku I. T etal. (2016) Tobacco Product Use Among Adults-United states, 2013-2014. MMWR Morb Mortal Wkly Rep, 65 , 685-691. doi: http://dx.doi.org/ 10.1 5585/mmwrmm6527al

Institute of Laboratory Animal Resources (2011). Guide for the Care and Use of Laboratory Animals. 8th ed. Institute of Laboratory Animals Resources, Commission of Life Sciences, National Research Council, Washington DC.

Jamal A., King B. A., Neff L. J., Whitmill J., Babb S. D, Graffunder C. M. (2016) Current Cigarette Smoking Among Adults -United States, 2005-2015. MM WR Morb Mortal Wkly Rep, 65, 1205-1211. doi: http://dx.doi.org/ 10.15585/mmwr.mm6544a2

Jones JD, Comer SD (2013) A review ofhuman drug self-administration procedures. Behav Pharmacol, 24(5-6): 38

Spindle, TR., Breland, A.B., Karaoghlanian, N.V., Shihadeh, A.L., & Eissenberg, T. (2015) Preliminary Results of an Examination of Electronic Cigarette User Puff Topography: The Effect of a Mouthpiece-Based Topography Measurement Device on Plasma Nicotine and Subjective Effects, Nicotine & Tobacco Research, 17, 142-149. http://doi.org/ 10.1093/ntr/ntu186

Can A, Dao DT, Arad M, Terrillion CE, Piantadosi SC, Gould TD (2012) The Mouse Forced Swim Test. JOVE 59: DOI 10.3791/3638

Dulawa SC, Holick KA, Gundersen B, Hen R (2004). Effects of Chronic Fluoxetine in Animal Models of Anxiety and Depression. Neuropsychopharmacology 29: 1321-1330.

Engin, E, Treit, D, Dickson, CT (2009). Anxiolytic- and antidepressant-like properties of ketamine in behavioral and neurophysiological animal models. Neuroscience 161: 359-369.

Ignatowsk-Jankowska BM, Muldoon PP, Lichtman AH, Damaj MI (2013) The cannabinoid CB2 receptor is necessary for nicotine-conditioned place preference, but not other behavioral effects of nicotine in mice. Psychopharmacology 229:591-601.

Institute of Laboratory Animal Resources (2011). Guide for the Care and Use of Laboratory Animals. 8th ed. Institute of Laboratory Animals Resources, Commission of Life Sciences, National Research Council, Washington DC.

Li N, Lee B, Liu R.-J, Banasr M, Dwyer JM, Iwata M (2010). mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. Science 329: 959-964.
Maeng, S, Zarate Jr, CA, Du, J, Schloesser, RJ, McCammon, J, Chen, G et al (2008). Cellular Mechanisms Underlying the Antidepressant Effects of Ketamine: Role of aAmino-3-Hydroxy-5-Methylisoxazole-4-Propionic Acid Receptors. Biol Psychiatry 63:349352.

Sunal, R, B, Kayaalp, SO (1994).Effect of changes in swimming area on results of "behavioral despair test". Pharmacology Biochemistry and Behavior 49: 891-896.

Talbot JN, Jutkiewicz EM, Graves SM, Clemans CF, Nicol MR, Mortensen RM et al (2010). RGS inhibition at Gai2 selectively potentiates 5-HTIA—mediated antidepressant effects. Proc Natl Acad sci U S A 107: 11086-11091.