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THE INFLUENCE OF ELECTRONIC CIGARETTE HEATING COIL RESISTANCE ON NICOTINE DELIVERY, HEART RATE, SUBJECTIVE EFFECTS, AND PUFF TOPOGRAPHY

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A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

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Table of Contents

Page

List of Tables

Page

List of Figures

Page

List of Abbreviations

ANOVA	analysis of variance
BP	blood pressure
CO	carbon monoxide
CReSS	Clinical Research Support System
ECIG	electronic cigarette
HR	heart rate
IRB	Institutional Review Board
IPI	Inter-puff-interval
LOQ	limit of quantification
min	minute(s)
mg	milligram
ng	nanogram (0.0000000001 grams)
ml	milliliter
ppm	concentration in parts per million
sec	second (s)
VAS	visual analog scale

Abstract

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Electronic cigarettes (ECIGs) are a class of products that aerosolize an often nicotine-containing solution for user inhalation. ECIG users can manipulate several device features including liquid nicotine concentration (mg/ml), battery voltage (V), and heating coil resistance (Ohms, Ω). One class of ECIG models, called "sub-Ohm" devices, use coils with a resistance of < 1 Ω, lower than those observed in conventional ECIGs (e.g., ≥ 1.5 Ω). Increasing voltage or decreasing coil resistance increases device power (Watts, or W). Limited clinical laboratory data suggest that high power devices deliver nicotine to the user effectively, even when paired with a low liquid nicotine concentration. Given that ECIG coil resistance and liquid nicotine concentration have not been manipulated systematically and simultaneously in clinical laboratory studies, the

influence of these factors on ECIG acute effects remain unclear. The primary purpose of this clinical laboratory study was to examine the influence of coil resistance and liquid nicotine concentration on nicotine delivery, heart rate (HR), subjective effects, puff topography, and liquid consumption.

Thirty-two experienced ECIG users completed four independent laboratory sessions that differed by coil resistance (0.5 Ω or 1.5 Ω; Kangertech SSOCC coils) and liquid nicotine concentration (3 or 8 mg/ml). In each session, participants used a 4.5 V "Kanger SUBOX" loaded with 3.5 ml ECIG liquid in a 10-puff directed (30 sec inter-puff interval) and 60-minute *ad libitum* bout. Broadly, coil resistance and liquid nicotine concentration had a direct influence on nicotine delivery, subjective effects, puff topography, and liquid consumption. Nicotine delivery was greatest when using 8 mg+0.5Ω combination and lowest when using the 3 mg/ml+1.5Ω combination and HR followed a similar pattern. Abstinence symptom suppression was most pronounced for the 8 mg+0.5Ω combination and least pronounced for the 3 mg/ml+1.5Ω combination. Participants provided the highest ratings for pleasantness, satisfaction, and liking of harshness/irritancy and throat hit sensations for the 3 mg+0.5Ω combination. Overall, use of ECIGs filled with 3 mg/ml nicotine concentration resulted in longer/larger puffs, increased puff frequency, and greater consumption of ECIG liquid. ECIG coil resistance, liquid nicotine concentration, and user puff topography, all of which influence ECIG nicotine delivery, should be considered together when making regulatory decisions aimed at protecting public health.

The Influence of Electronic Cigarette Heating Coil Resistance on Nicotine Delivery, Heart Rate, Subjective Effects, and Puff Topography

Overview

Tobacco cigarette smoking remains the leading preventable cause of death and attributes to 480,000 deaths per year in the U.S. (USDHHS, 2014) and an estimated 6 million deaths per year globally (Agaku et al., 2014). Numerous negative health consequences such as various cancers, cardiovascular disease, and stroke are caused by tobacco cigarette consumption (Mathers & Loncar, 2006). The financial costs of smoking-related morbidity in the U.S. exceed $300 billion each year in direct medical costs and loss of productivity (USDHHS, 2014; Xu, Bishop, Kennedy, Simpson, & Pechacek, 2015). Despite the health threat of cigarette smoking, approximately 14% of U.S. adults and 8.1% of high school students continue to smoke (Cullen et al., 2018; Gentzke et al., 2019; Wang et al., 2018). Smoking cessation is difficult primarily because regular self-administration of cigarette-delivered nicotine, a psychoactive tobacco constituent, causes nicotine/tobacco dependence (e.g., Benowitz, 2008; Stratton, Shetty, Wallace & Bondurant, 2001). In addition to cigarettes, several other nicotine-containing tobacco products have emerged in U.S. and global markets. One such tobacco product, the electronic cigarette (ECIG), has become increasingly popular and is now the preferred tobacco product among youth (Gentzke et al., 2019).

ECIGs electrically heat a liquid solution, that often contains nicotine, and produce an aerosol for users to inhale. ECIG components typically include a power source such as a battery, an electrical heating element or coil, and a reservoir that contains a liquid solution composed of solvents, flavors, and oftentimes nicotine (Breland et al., 2017; Etter, 2012). Despite several common features (e.g., battery, coil, and liquid) ECIGs vary considerably in their device features, many of which can be modified by the user. The variability in ECIG device and liquid

features can be attributed to that fact that, until recently, ECIGs were unregulated in the U.S. However, in May of 2016 the U.S. Food and Drug Administration (FDA) announced its intention to regulate ECIGs (Deeming Tobacco Products To Be Subject to the Federal Food, Drug, and Cosmetic Act, as Amended by the Family Smoking Prevention and Tobacco Control Act, 2016; hereafter "Deeming, 2016"). Although the acute effects of some specific ECIG brands/models have been evaluated (i.e., nicotine delivery and subjective effects; Dawkins, Kimber, Doig, Feyerabend, & Corcoran, 2016; Nides, Leischow, Bhatter, & Simmons, 2014; Vansickel & Eissenberg, 2013), the rapidity with which these products evolve necessitates further evaluation to facilitate appropriate regulatory decisions. Effective regulation requires an understanding of the individual and combined influence of ECIG device and liquid features on outcomes of interest.

The goal of this dissertation is to use established clinical laboratory methodology to evaluate the influence of ECIG heating coil resistance and liquid nicotine concentration on outcomes predictive of dependence (i.e., nicotine delivery and subjective effects) while evaluating user puffing behavior. The review below describes ECIG prevalence and use patterns, the variability in ECIG device and liquid characteristics, ECIG regulation, pre-clinical evaluations of ECIGs and the results of several clinical laboratory evaluations of ECIGs. The review will conclude by describing an emerging class of ECIG devices that require empirical investigation.

ECIG Use Patterns and Reasons for Use

ECIGs were patented in 2003 by Hon Lik (Lik, 2003) and were released to the U.S. market in 2007. Since their introduction to U.S. and global markets, ECIG use prevalence has been increasing (Wang et al., 2018; Cullen et al., 2018; Jamal et al., 2017) as have marketing and

advertising expenditures to promote ECIGs (Kornfield, Huang, Vera, & Emery, 2015; Giovenco, Hammond, Corey, Ambrose, & Delnevo, 2015). In the U.S., ECIGs are subject to few marketing restrictions and consequently are advertised in magazines/newspapers, on television, radio, in retail stores, and on the internet which includes social media platforms (Collins, Glasser, Abudayyeh, Pearson, & Villanti, 2019; Link, Cawkwell, Shelley, & Sherman, 2015). Many marketing strategies used to promote ECIGs appeal to youth (i.e., celebrity endorsements, themes of rebellion, freedom, and relaxation) and consequently have been prohibited in cigarette marketing for decades (Duke, Lee, Kim, Watson, Arnold, Nonnemaker, & Porter, 2014; England, Bunnell, Pechacek, Tong, & Mcafee, 2015; Marynak, Gentzke, Wang, Neff, & King, 2018; USDHHS, 2016, 2012). ECIGs also have been marketed as smoking cessation aids and as less harmful alternatives to tobacco cigarettes (Willis, Haught, & Morris, 2017; Collins et al., 2019). ECIG accessibility has also increased among traditional tobacco retailers (i.e., convenience stores and gas stations) and retail outlets called "vape shops" that specialize in the sale of ECIGs (Burbank, Thrul, Ling, 2016; Dai & Hao, 2017; Hahn, Begley, Gokun, Johnson, Mundy, & Rayens, 2015; Lee & Kim, 2015). Of particular concern are the increasing number of ECIG retail outlets, many of which are in close proximity to schools and universities (Dai & Hao, 2017; Hahn et al., 2015; Wagoner et al., 2014). In 2015, 9,945 "vape shops" were identified in the U.S., a near threefold increase from 2013 (Dai & Hao, 2017). Investigators also identified that among 2,775 U.S. colleges, 66.5% were within a 3-mile radius of at least one "vape shop" (Dai & Hao, 2017). Unrestricted ECIG marketing practices and increases in ECIG accessibility have been accompanied by increases in ECIG use prevalence among adults and adolescents in the U.S.

ECIG use among adults. Since 2010, overall ECIG use prevalence among U.S. adults has increased as evidenced by patterns of ever use (i.e., lifetime use) and current use (i.e., past 30-day use) of these products. In a study of four cross-sectional, nationally representative samples, ever use of ECIGs among U.S. adults increased from 1.8% in 2010 to 13% in 2013 (McMillen, Gottlieb, Shaefer, Winickoff, and Klein, 2015). More recent examinations of national data from the 2017 National Health Interview Survey of 26,742 U.S. adults (≥ 18 years) reveal that an estimated 2.8% (6.9 million) report current ECIG use (defined as using ECIGs every day or some days; Wang et al., 2018). A more detailed examination of the data revealed that current use of ECIGs among adults is highest among the following groups: males, young adults aged 18-24, and Caucasians. Furthermore, several reports reveal that among U.S. adults who use ECIGs currently, the majority are current or former cigarette smokers (Coleman et al., 2017; Delnevo et al., 2016; Mirbolouk et al., 2018; Rodu & Plurphanswat, 2017). Among U.S. adults, the most commonly cited reasons for using ECIGs are to quit smoking or to reduce consumption of tobacco cigarettes (Berg, Haardoerfer, Escoffery, Zheng, & Kegler, 2015; Patel et al., 2017; Richardson, Pearson, Xiao, Stalgaitis, & Vallone, 2014; Soneji et al., 2019; Soule, Rosas, & Nasim, 2016). For example, among a national sample of 2,448 adults who currently use ECIGs 84.5% reported using these products for reducing smoking related harm and for assistance with smoking cessation/reduction (Patel et al., 2017).

ECIG use among adolescents. According to data from the National Youth Tobacco Survey (NYTS), a cross-sectional school-based survey administered to U.S. middle and high school students, from 2014 to 2018, ECIGs are the most popular tobacco product among this population (Gentzke et al., 2019). These data also reveal substantial increases in ECIG use, such that from 2011 to 2018 current ECIG use increased from 1.5% to 20.8% among high school

students and from 0.6% to 4.9% among middle school students (Gentzke et al., 2019). These data correspond to 3.05 million high school students and 570,000 middle school students who report current ECIG use (Gentzke et al., 2019). Unlike adult ECIG users, adolescent ECIG users do not cite smoking cessation or reducing smoking related harm as their primary reasons for ECIG use (Tsai et al., 2018). Data from the NYTS indicate that middle and high school students report peer and parental ECIG use, availability of flavors, and the belief that ECIGs are less harmful than cigarettes as primary reasons for using ECIGs (Tsai et al., 2018).

ECIG use among nicotine-naïve individuals. Of particular concern are the number of formerly nicotine-naïve adults and adolescents who use ECIGs. Individuals who are nicotine-naïve have never initiated nicotine/tobacco use and therefore are not nicotine dependent. According to data from the national 2013-2014 Population Assessment of Tobacco and Health (PATH) survey, 16% of U.S. adults who report current ECIG use are never smokers (Coleman et al., 2017). Of these adult ECIG users who have never smoked, 8.5% report using ECIGs daily (Coleman et al., 2017). Moreover, data from the 2014 National Health Interview Survey reveal that 9.7% of young adults aged 18-24 who have never smoked cigarettes report having tried ECIGs (Schoenborn & Gindi, 2015). More recently, data from the national 2016 Behavioral Risk Factor Surveillance System indicate that among young adults aged 18-24 who report current ECIG use, 44% (to 1.2 million) had never initiated tobacco cigarette smoking (Mirbolouk et al., 2016). Numbers of nicotine-naïve adolescents who use ECIGs also are growing. Among U.S. adolescents, never smokers who report ever use of ECIGs increased from 79,000 in 2011 to 263,000 in 2013 (Bunnell et al., 2014). Among 482,179 middle and high school students who participated in the 2013-2014 California Healthy Kids Survey, 60,000 had used ECIGs despite never smoking cigarettes (Bostean, Trinidad, & McCarthy, 2015). ECIG use among never

smoking adolescents, young adults, and adults is also reported in numerous regional examinations of ECIG use (Barrington-Trimis et al., 2016, 2018; Chapman & Wu, 2014; Hammet, Veldheer, Yingst, Hrabovsky, & Foulds, 2017; Leventhal et al., 2015; Spindle et al., 2017; Wills, Knight, Sargent, Gibbons, Pagano, & Williams, 2016).

The use of ECIGs among formerly nicotine-naïve youth and young adults raises concerns regarding the possibility that ECIG use may increase the likelihood of subsequent initiation of tobacco cigarettes. Indeed, according to numerous longitudinal investigations, adolescent and young adult ECIG users are more likely to initiate cigarette smoking after having used ECIGs (Barrington-Trimis et al., 2018; Leventhal et al., 2015; Spindle et al., 2017; Unger, Soto, & Leventhal, 2016; Wills et al., 2016). A 3-wave (2013, 2014, and 2015) longitudinal survey administered to high school students in Connecticut revealed that current use of ECIGs at time 1 predicted future cigarette use at time 2 and 3 (Bold et al., 2017). Despite the association between ECIG use and subsequent cigarette smoking, current use of cigarettes did not predict subsequent ECIG initiation (Bold et al., 2017). Furthermore, a meta-analysis of nine longitudinal studies indicated ECIG use was associated with a 3-fold increased risk for future cigarette smoking even after adjusting for factors that predict cigarette use independently (i.e., demographic, psychosocial and behavioral risk factors; Soneji et al., 2017). While the mechanisms that drive the transition from ECIG use to cigarette use are unclear, such a transition could have negative individual and population level health consequences.

Taken together, since ECIGs emerged on the U.S. market, they have become increasingly popular among adults and adolescents including individuals from both groups who formerly were naïve to tobacco/nicotine. However, understanding the public health impact of ECIGs will require more than characterizing who uses these products. To date, many epidemiology studies

examining patterns of and reasons for ECIG use refer to ECIGs as a single product rather than differentiating between the numerous ECIG models and liquids available on the market. Future examinations assessing the extent to which use patterns differ across different ECIG devices and liquids are necessary because as described below, several ECIG device and liquid characteristics vary substantially and can influence the content of the aerosols emitted from ECIGs and the acute effects those aerosols have on the user.

Characteristics of Electronic Cigarettes

ECIGs generally contain a power source (e.g., battery), an electrical heating coil (called an "atomizer" by ECIG users), and a reservoir that stores a liquid solution (composed of solvents, flavors, and, oftentimes, nicotine). Despite common general features (i.e., power source, heating coil and liquid solution) ECIGs vary widely in terms of appearance, user-controlled device features, and liquid constituents. Given this variability, ECIGs are often referred to as a product class or category, rather than a single device (Breland et al., 2017).

Device Characteristics. With over 466 different ECIG brands on the market, ECIG models vary considerably in terms of appearance (Zhu, Sun, Bonnevie, Cummins, Gamst, Yin, & Lee, 2014). Early ECIG models were designed to resemble the size and shape of a traditional tobacco cigarette and are often referred to as "cigalikes" or "first-generation devices" (see Figure 1; Etter, 2012; Breland et al., 2017). "Cigalike" models often contain a light emitting diode that glows at the non-mouth end of the device as a result of inhalation. These models store a liquid solution in cartridges (referred to as "cartomizers"). Following some period of use, the cartridge or "cartomizer" is depleted of liquid and depending on the "cigalike" model, the user is required to replace, refill, or dispose of the cartridge entirely (Breland et al., 2014; Etter, 2012). Recently, devices have emerged on the market that operate under similar principles (i.e., they include a

disposable cartridge that must be replaced) but do not resemble the size and shape of a cigarette (e.g., "JUUL"; Giroud, de Cesare, Berthet, Varlet, Concha-Lozano, & Favrat., 2015).

Aside from "cigalikes," numerous other ECIG models that are not intended to resemble cigarettes are available. These other ECIG models contain non-disposable, rechargeable batteries and contain liquid in prefilled cartridges or in refillable reservoirs, called "tanks" (see Figure 1; Etter, 2012). Users may refill "tanks" as needed by purchasing refill solution. Moreover, these tanks can vary in size and capacity such that some ECIGs tanks can store upwards of 10 milliliters (ml) of ECIG liquid, much more than the typical cartomizer that stores 0.5-2 mls (Etter, 2012). Often these models are activated manually when the user presses a button on the mouth end of the device (Breland et al., 2014; Etter, 2012).

In addition to varying in terms of appearance, ECIG models can differ with regard to several device characteristics that include the voltage (measured in Volts; V) of the power supply and/or heating coil resistance (measured in Ohms or Ω; Etter, 2012). Together these two factors influence the overall electrical power output of the device (measured in watts; W) such that $W=V^2/\Omega$. ECIG battery voltage refers to the force with which an electrical current runs through a heating coil and can range from 3 to 6 V, though battery voltage as high as 14.7 V has been reported (Rudy et al., 2017). The heating coil of an ECIG is typically composed of one or multiple metallic coils that serve as an interal heating element (Breland et al., 2017). When the user inhales and/or presses a button to activate the device, the coil is activated, heats a liquid and subsequently produces aerosol. While conventional ECIG models operate with coil resistance ranging from 1.0-6.5 Ω, recent models that operate with coil resistance <1 Ω have emerged (Breland et al., 2017; Talih et al., 2017b). Given the inverse relationship between device power and coil resistance, ECIGs with low resistance coils can operate at much higher power (e.g., 50-

300 W; Talih et al., 2017b) relative to conventional devices. Indeed, in one study, when coil resistance ranged from 0.1-0.6 Ω, device power ranged from 18.6-162.4 W (Wagener et al., 2017). As will be described in detail below, device power can alter the content of toxicant emissions that emerge from the mouth end of the device (Kosmider, Sobczak, Knysak, Goniewicz, 2014; Talih et al., 2015; 2017a).

ECIG Liquids. As with ECIG device characteristics, ECIG liquid solutions vary substantially. These solutions are often referred to as "e liquid" or "juice" and are typically composed of flavorants, a liquid vehicle (or solvent), and, oftentimes, nicotine.

Flavorants: An estimated 7,700 unique characterizing flavors are available on the market for use with ECIGs (Zhu et al., 2014). In an effort to protect public health, in 2009, the U.S. passed legislation that banned the use of characterizing flavors in tobacco cigarettes with "tobacco" and "menthol" flavor being the two exceptions (Family Smoking Prevention and Tobacco Control Act, 2015). However, ECIGs are not subject to these flavor restrictions and continue to be available commercially with a wide variety of characterizing flavors in categories such as fruit, candy, dessert, and drink as well as tobacco and menthol flavor (Dawkins et al., 2013; Farsalinos et al., 2013). These flavored ECIG liquids are used widely (Berg et al., 2014; Harrell et al., 2017; Soneji et al., 2019; Villanti et al., 2017). For example, data from the 2013-2014 PATH survey reveal that among current ECIG users, 85.3% of youth and 83.4% of adults report current use of flavored ECIGs (Villanti et al., 2017). Furthermore, a 2014-2015 cross-sectional examination of youth and college students in Texas as well as young and older adults nationwide revealed that the majority of current ECIG users reported using an ECIG in the past 30 days that consisted of a flavor other than tobacco (Harrell et al., 2017). Across all age groups, current ECIG users reported fruit and candy flavors as being preferred predominantly (Harrell et

al., 2017). These results are supported by data from an online survey of ECIG users revealing that commonly used ECIG flavor categories include fruit and sweet, followed by tobacco flavor (Farsalinos et al., 2013). Importantly ECIG users cite the availability of a wide variety of flavors as a reason for ECIG use (Berg et al., 2014; Farsalinos et al., 2013b; Kong et al., 2015; Soule et al., 2016; Soneji et al., 2019) and self-report that ECIG flavors increase satisfaction and enjoyment during use (Soule et al., 2016).

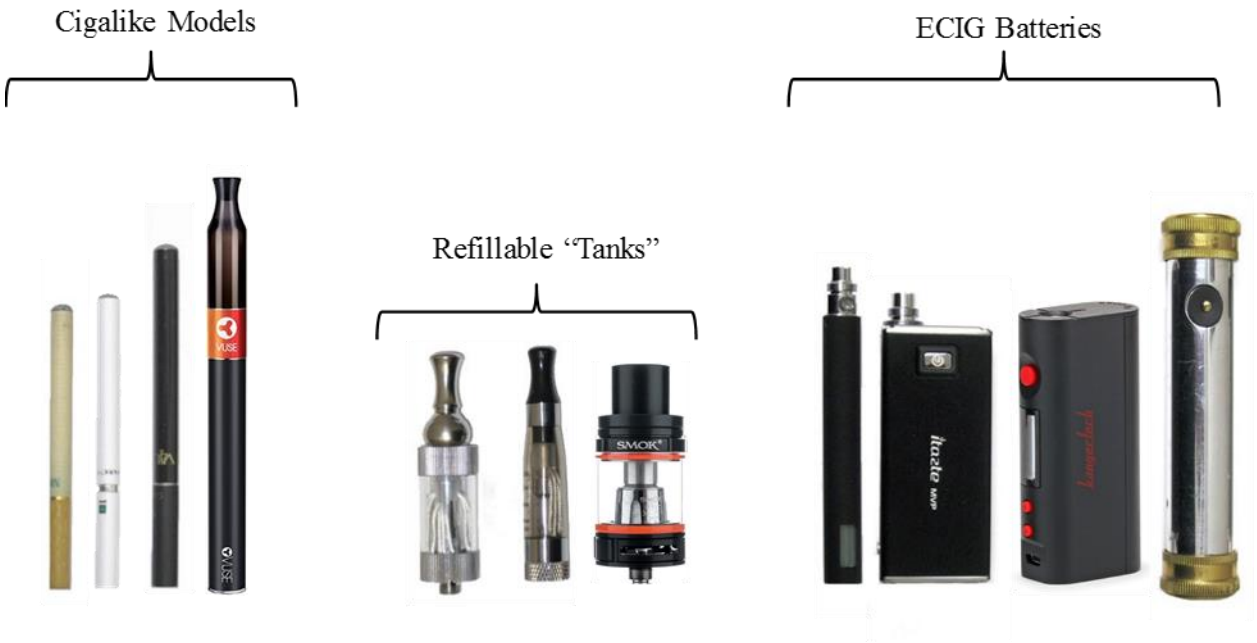

Figure 1. From the left, "cigalike" ECIG models that store ECIG liquid in pre-filled cartridges (i.e. "cartomizers"), refillable ECIG "tanks", and rechargeable ECIG batteries.

Solvents: One of the main ingredients in ECIG liquids is the solvent in which nicotine and flavorants are dissolved. The two solvents most commonly used in ECIG liquids are propylene glycol (PG) and vegetable glycerin (VG; Chen, Zhang, Liu, & Fu, 2015). As much as 95% or more of the ECIG liquid content can be comprised of PG and/or VG (Chen et al., 2015). The ratio of PG to VG in ECIG liquid can range anywhere from 0:100 or 100:0 (Etter, 2012). When PG and/or VG are heated and aerosolized, they act, alone or in combination, as the vehicle to carry nicotine and/or flavorants to the user (Etter, 2012). Further, solvent ratio is one factor that influences nicotine yield (Kosmider et al., 2018) and delivery to users (Spindle et al., 2018).

Nicotine: The nicotine concentration of ECIG liquids ranges usually from 0-36 mg/ml, though products with nicotine concentrations much higher (e.g., 50+ mg/ml) are available commercially (Breland et al., 2014; Ramamurthi, Chau, & Jackler, 2018; Varlet, Farsalinos, Augsburger, Thomas, & Etter, 2015). One concern regarding the content of ECIG liquid nicotine concentration is the inconsistency between actual and advertised nicotine concentrations observed in ECIG liquids (Bahl et al., 2012; Trehy et al., 2011). That is, some ECIG liquids advertised as containing no nicotine (0 mg/ml) contain trace levels of nicotine (Hadwiger et al., 2010; Trehy et al., 2011; Kubica et al., 2013). Also, some ECIG liquids are mislabeled such that the liquid contains nicotine at much lower concentrations than what is advertised (Goniewicz, Kuma, Gawron, Knysak, & Kosimider, 2013; Spindle, Breland, Karaoghlanian, Shihadeh, & Eissenberg, 2015). For example, the liquid nicotine concentration stored in the cartridges of six popular United Kingdom ECIG brands was analyzed to explore labeling inconsistencies and variation in liquid nicotine concentration of up to 12% was observed (Goniewicz, Hajek, McRobbie, 2012). Further, in one clinical laboratory study involving experienced ECIG users, two participants brought liquid into the laboratory that they used regularly, labeled “12 mg/ml,”

but subsequent analysis revealed that the liquid contained no measurable nicotine (Spindle et al., 2015).

The variability of ECIG device and liquid characteristics can be attributed largely to the absence of regulations for this product class in many countries, including the U.S. Currently ECIGs are unregulated in many countries though some jurisdictions have begun to regulate ECIG liquids. For example, the European Union limits ECIG liquid such that liquid nicotine concentration cannot exceed 20 mg/ml (European Union Directive, 2014/40/EU). In August 2016, the U.S. FDA announced its intention regulate ECIGs and their "parts and components" (Deeming, 2016).

Regulation: Tobacco Control Act

In June of 2009, The Family Smoking Prevention and Tobacco Control Act (or Tobacco Control Act; TCA) was enacted and gave FDA regulatory authority over the manufacturing, distribution and marketing of certain tobacco products (Family Smoking Prevention and Tobacco Control Act, 2009). Products that were covered immediately by FDA's tobacco product authority included cigarettes, cigarette tobacco, roll-your-own tobacco, and smokeless tobacco. The Tobacco Control Act also granted FDA the authority to regulate other products that were not covered under the rule initially and that meet FDA's definition of a tobacco product. FDA defines tobacco products as "any product made or derived from tobacco that is intended for human consumption, including any component, part or accessory of the tobacco product" (Deeming, 2016). Notably, the definition does not include tobacco products that are "marketed for therapeutic purposes."

In May of 2016, the FDA announced its regulatory authority would extend to include ECIGs and other novel tobacco products (Deeming, 2016). ECIGs meet FDA's definition of a

tobacco product because they contain nicotine derived from the tobacco plant, are "intended for human consumption," and they are not intended for therapeutic purposes. The FDA's regulatory authority over ECIGs will include general controls such as registration of products, listing of product ingredients, provisions against adulteration and misbranding, and premarket review of new tobacco products (Deeming, 2016). To protect public health, FDA's new regulatory authority asserts that manufacturers of novel tobacco products, such as ECIGs, must obtain authorization from FDA in order to market their products in the U.S. One way for ECIG manufacturers and retailers to obtain FDA authorization for new products is to submit a premarket tobacco product application containing information such as details regarding product components and ingredients, additives, labeling information as well as health risk information. Through this application process, FDA intends to ensure that only products that demonstrate appropriate protections for public health will be allowable on the market. For the purpose of product premarket applications, FDA considers each product, with different flavorants or liquid nicotine concentrations to be a different product.

FDA's authority over ECIGs could protect public health in several ways. For example, FDA oversight of ECIG cartridges and ECIG liquids may reduce the concentration of chemicals present in ECIG liquids and ensure that the chemicals present are labeled accurately (Deeming, 2016). Further, premarket review of ECIGs and their liquids may increase consistency across products with regard to the type of chemicals and concentrations of chemicals being aerosolized and inhaled by users. Also, enforcing a minimum age requirement for newly deemed tobacco products such as ECIGs may reduce youth access and dependence on nicotine containing products. As such, retailers are prohibited from selling ECIGs to individuals under 18 years of age through any medium, including the internet.

To enforce effective tobacco-related policies, regulatory agencies will rely on premarket tobacco product applications and empirical evidence from tobacco scientists to generate information regarding what these products do, what ingredients they contain, and the extent to which they foster nicotine/tobacco dependence. More specifically, to understand the dependence potential ECIGs, regulatory agencies will require an understanding of nicotine dose and delivery in addition to other ECIG acute effects (i.e., suppression of abstinence symptoms; Carter, Stitzer, Henningfield, O'Connor, Cummings, & Hatsukami, 2009). For inhalable tobacco products such as ECIGs, nicotine dose is a function of nicotine yield, or the amount of nicotine present in ECIG emissions, whereas nicotine delivery is the amount of nicotine that is ultimately delivered to the user's blood (often measured in blood plasma; Connolly, Alpert, Wayne, & Koh, 2007; Land, Keithly, Kane, Chen, Paskowsky, Cullen, Hayes, & Li, 2014). In addition to these factors, understanding whether ECIGs can produce subjective effect profiles capable of producing and maintaining nicotine dependence (e.g., producing pleasurable effects after use and alleviating aversive nicotine abstinence symptoms, such as irritability and anxiety) will be important (Carter et al., 2009; Eissenberg, 2004; Glautier, 2004). The review below focuses on how various ECIG device and liquid characteristics influence ECIG emissions and ECIG acute effects (i.e., nicotine delivery and subjective effects such as abstinence symptom suppression). Also described below is the influence of user puffing behavior (i.e., puff topography) including puff number, puff duration (measured in seconds), puff volume (ml), IPI, and flow rate (ml/sec) on these outcomes.

Pre-Clinical Evaluation of ECIGs: Nicotine Yield

Emissions from ECIGs, such as nicotine and other toxicants present in the aerosol, have been examined in numerous pre-clinical laboratory studies (Baassiri et al., 2017; El-Hellani et al., 2016; Kosmider et al., 2014; Kosmider, Spindle, Gawron, Sobczak, & Goniewicz, 2018;

Ogunwale et al., 2017; Pankow et al., 2017; Soussy, El-Hellani, Baalbaki, Salman, Shihadeh, & Saliba, 2016; Talih et al., 2015; Talih et al., 2017a). In these pre-clinical studies, investigators program machines to puff on an ECIG and analyze the content of the aerosol produced to understand the amount of nicotine and other toxicants that a user might inhale. For combustible cigarettes, nicotine yield, defined as the mass of nicotine emitted from the mouth end of a cigarette (measured in milligrams; mg), historically has been used to characterize nicotine emissions (Connolly et al., 2007; Land et al., 2014). Similarly, nicotine yield has been a valuable tool for understanding how ECIG device and liquid features as well as user puffing behavior influence nicotine emissions.

Altering ECIG design features such as battery voltage and coil resistance (which together influence power) can influence ECIG nicotine yield (Kosmider et al., 2014; Talih et al., 2015). In one analytical laboratory study, puffing machines were programmed to generate aerosols from ECIGs while battery voltage was manipulated to 3.3 V or 5.2 V and coil resistance (3.6 Ω), puff duration (4 sec), flow rate (17 ml/sec) and nicotine concentration (8.53 mg/ml) were held constant (Talih et al., 2015). Under these controlled conditions, after 15 puffs mean (SD) aerosol nicotine yield increased from 0.3 mg (0.01) at 3.3 V to 1.2 mg (0.3) at 5.2 V (Talih et al., 2015). Similarly, in another study, investigators manipulated battery voltages to 3.2, 4.0, and 4.8 V while several puffing parameters (15 puffs of 2 sec puff duration, 17 sec IPI, 50 ml puff volume, and 25 ml/sec flow rate), ECIG liquid nicotine concentration (18 mg/ml), and liquid solvent (PG) were held constant (Kosmider et al., 2014). Following 15 puffs, mean (SD) aerosol nicotine yield was 0.9 mg (0.2) at 3.2 V, 1.0 mg (0.3) at 4.0 V, and 1.3 mg (0.4) at 4.8 V (Kosmider et al., 2014). Collectively, these studies demonstrate that increases in device voltage correspond with increases in nicotine yield. Therefore, increases in overall device power by means of increasing

battery voltage or decreasing coil resistance can result in increases in ECIG nicotine yield (Kosmider et al., 2014; Talih et al., 2015).

Altering the characteristics of ECIG liquid, such as manipulating liquid nicotine concentration or PG:VG ratio can also influence nicotine yield (El-Hellani et al., 2016; Kosmider et al., 2014; Talih et al., 2015; Talih et al., 2017a). With regard to liquid nicotine concentration, several studies have been conducted to compare nicotine yield across different ECIG brands containing various nicotine concentrations (Goniewicz, Kuma, Gawron, Knysak, & Kosmider, 2013; Laugesen, 2015; Tayyarah & Long, 2014), although only a few studies have manipulated liquid nicotine systematically while holding other relevant device and liquid features constant (Talih et al., 2015, Talih et al., 2017a). In one such study, ECIG liquid nicotine concentration was either 8.53 mg/ml or 15.73 mg/ml while voltage (5.2 V), puff duration (8 sec), and flow rate (17 ml/sec) were held constant (Talih et al., 2015). As liquid nicotine concentration increased, so did nicotine yield such that after 15 puffs, mean (SD) nicotine yield was 3.2 mg (0.3) for the 8.53 mg/ml condition and 4.7 mg (1.0) for the 15.73 mg/ml condition. In another study, the influence of PG:VG ratio and battery voltage were evaluated to determine how these factors affect the nicotine yield of an ECIG (Kosmider et al., 2018). Ratio of PG:VG (0:100, 50:50, 100:0) and battery voltage (3.2 V or 4.8 V) were manipulated while puff topography variables (15 puffs of 2 sec puff duration, 17 sec IPI, 50 ml puff volume, and 25 ml/sec flow rate) and ECIG liquid nicotine concentration (18 mg/ml) were held constant. Overall, as the concentration of the PG in the liquid increased, so did nicotine yield albeit only when using the low power setting. That is, when battery voltage was set to 3.2 V, mean (SD) nicotine yield was 0.46 mg (0.08) in the 100 VG condition, 0.59 mg (0.14) in the 50VG:50PG condition, and 0.85 mg (0.16) in the 100 PG condition (Kosmider et al., 2018). Finally, in one pre-clinical study, nicotine yield was examined

across four different PG:VG ratios (100VG, 80VG:20PG, 50PG:50VG, 100PG) while device power (4.3 W), puff topography variables (15 puffs, 4 sec puff duration, 10 sec IPI, 66.7 ml puff volume, and 16.7 ml/sec flow rate) and liquid nicotine concentration (18 mg/ml) were held constant (Baassiri et al., 2017). The mean (SD) nicotine yield was 0.13 mg (0.02) in the 100VG condition, 0.17 mg (0.01) in the 20PG:80VG condition, 0.33 mg (0.01) in the 50PG:50VG condition and 0.58 mg (0.02) in the 100PG condition (Baassiri et al., 2017). As such, when all other factors are held constant, increases in the amount of PG in ECIG liquid result in greater nicotine yield. Collectively, results from these studies are consistent with the notion that some aspects of ECIG liquid composition such nicotine concentration and PG:VG ratio can influence ECIG nicotine yield.

Finally, puff topography variables also influence nicotine yield (Talih et al., 2015; Talih et al., 2017a). In one of the aforementioned studies, investigators analyzed nicotine yield after altering various puff topography variables while holding device voltage (3.3 V, which converts to 3.0 W when using a 3.6 Ω coil) and the ECIG liquid nicotine concentration (8.53 mg/ml) were held constant. Specifically, ECIG aerosols were generated using hardware and software that could simulate the distinct puffing profiles of various types of tobacco cigarette and ECIG users based on their puff duration, puff volume, and flow rate. For example, the puffing profile for a tobacco cigarette smoker included 2 sec duration puffs with 66 ml puff volume, at 33 ml/s flow rate, fast average ECIG user puffs were 4 sec, 132 ml at 33 ml/s, and slow extreme ECIG user puffs were 8 sec 136 ml at 17 ml/s. Results indicated that nicotine yield from 15 puffs varied across individual puffing profiles such that nicotine yield was lowest for puffs that simulated a tobacco cigarette smoker relative to the two puffing profiles that simulated experienced ECIG users (Talih et al., 2015). After 15 puffs mean (SD) aerosol nicotine yield for tobacco cigarette

smokers puffing profiles was 0.11 mg (0.02) while for ECIG user puffing profiles nicotine yield was 0.29 mg (0.08) for fast average ECIG users and 0.72 mg (0.1) for slow extreme ECIG users (Talih et al., 2015). Overall, these results indicate that longer puff durations are associated with increases in aerosol nicotine yield, although increases in flow rate have a minimal effect on nicotine yield (Talih et al., 2015). One reason longer duration puffs correspond with increases in nicotine yield is because during longer duration puffs, the ECIG heating coil is activated for a longer period of time resulting in a larger proportion of the puffing time spent in a higher-temperature phase (Talih et al., 2015). Puffing during the higher temperature phase results in higher nicotine evaporation rates, thus leading to greater nicotine yield. Conversely puff velocity does not increase nicotine yield (Talih et al., 2015). While increases in puff velocity increase the ability for air flowing through the device to collect nicotine vapor from the surface of the coil, these increases in puff velocity also decrease coil temperature resulting in lower nicotine evaporation. Together these two factors counteract one another and explain why puff velocity does not influence nicotine yield.

Collectively, results from systematic pre-clinical analytical laboratory studies reveal that, all other factors being equal, increasing device voltage, the concentration of nicotine or PG in the liquid, and puff number or duration will increase the amount of nicotine present in the aerosol emitted from an ECIG (Baassiri et al., 2017; El-Hellani et al., 2016; Kosmider et al., 2014, 2018; Talih et al., 2015; Talih et al., 2017a). Despite the importance of these findings, nicotine yield does not necessarily represent the amount of nicotine delivered to the user's blood, highlighting the need to conduct human clinical laboratory studies to expand upon the pre-clinical findings.

Clinical Laboratory Evaluations of ECIGs

Clinical laboratory studies have been conducted to examine acute effects following ECIG

use including the amount of nicotine detected in the user's blood, heart rate, and subjective experience. To date, ECIG acute effects have been measured in numerous clinical laboratory studies typically with within-subject study designs (e.g., Dawkins & Corcoran, 2014; Dawkins et al., 2016, 2018; Farsalinos et al., 2014; Maloney et al., 2019; Spindle et al., 2015, 2017, 2018; Vansickel & Eissenberg, 2013) and with participants who are either experienced ECIG users or ECIG-naïve cigarette smokers. Notably, findings from clinical laboratory studies reveal that ECIG acute effects are influenced by factors such as device features, liquid constituents, and user puffing behavior.

When assessing ECIGs, many clinical laboratory study protocols and methodologies have been modeled after those historically used to study the effects and use behavior associated with tobacco cigarettes. For example, several ECIG studies include a "directed" ECIG use bout whereby participants are instructed to take a specified number of puffs over a fixed period of time (Farsalinos et al., 2015; Hiler et al., 2017; Spindle, 2018; Vansickel et al., 2010; Vansickel & Eissenberg, 2013), as has been done for tobacco cigarettes and other combustible tobacco products (Blank, Nasim, Hart, & Eissenberg, 2011; Griffiths, Henningfield, & Bigelow, 1982). Other ECIG studies include *ad libitum* ECIG-use bouts where participants are instructed to puff on an ECIG as often as they like during a set period of time (e.g., Farsalinos et al., 2014; Vansickel & Eissenberg, 2013), as has been done for tobacco cigarettes and other combustible tobacco products (Breland, 2005; Blank, 2009; Gust & Pickens, 1982; Herning et al., 1983; Rose & Behm, 2003; Wall et al., 2018). As will be described below, when assessing novel tobacco products directed use and *ad libitum* bouts each have unique advantages.

The use of standardized puffing protocols when evaluating novel tobacco products has become commonplace (Breland, Buchhalter, Evans, & Eissenberg, 2002; Blank, Nasim, Hart, &

Eissenberg, 2011; Lopez, Hiler, Maloney, Eissenberg, & Breland, 2016). Often, the puffing behavior that is standardized includes puff count (i.e., the number of puffs taken) and IPI (defined as the time between the onset of one puff and the onset of a subsequent puff; as in Farsalinos et al., 2014; Hiler et al., 2017; Lopez et al., 2016; Spindle et al., 2015, 2017; Vansickel et al., 2010). Standardization of tobacco use behavior in the clinical laboratory has two advantages. First, standardizing puffing behavior allows investigators to draw comparisons across different tobacco products (e.g., tobacco cigarettes versus ECIGs) when the same protocol is followed for both products. Second, standardized puffing protocols (i.e., 10 puffs; 30 sec IPI) are reflective of actual use behaviors of a population that is often of interest to investigators (e.g., cigarette smokers). Data from cigarette smokers support the notion that a 10-puff bout is typical during the consumption of a single tobacco cigarette. That is, in a within-subject design study intended to compare three different techniques for measuring puffing behavior (desktop, portable, or video method), cigarette smokers using their own brand cigarettes *ad libitum* took the following mean (SD) puff numbers: 9.7 puffs (3.3) with the desktop topography measurement system, 9.4 puffs (3.0) with the portable measurement system, and 9.2 puffs (3.2) with the video method recording (Blank, Disharoon, & Eissenberg, 2009). However, measurement of IPI in this study revealed that 18 sec IPI was the norm. These results support the use of a 10-puff directed bout, but do not support 30 sec IPI (see also Kleykamp, Jennings, Sams, Weaver, Eissenberg, 2008 for similar support for 10 puffs but not for 30 sec IPI). However, in both studies, participants were overnight tobacco abstinent, which may have influenced user puff topography.

In another study, involving 36 non-abstinent smokers, participants completed four, 5-day conditions that differed by product, with one product being the participants' own brand tobacco

cigarette. Laboratory measurement of *ad libitum* puff topography was conducted on days 1 and 5 of each condition. In the own brand condition, mean (SD) puff number was 10 puffs (3.1) on day 1 and 10 puffs (2.7) on day 5 (Breland, 2005), providing support for the notion that a mean of 10 puffs is typical for a tobacco cigarette smoker smoking a single cigarette. Moreover, mean (SD) IPI was 30.7 sec (12.8) on day 1 and 30.5 sec (9.6) on day 5 (Breland 2005). This study, that included tobacco cigarette smokers who had not abstained from cigarettes prior to puff topography measurement, may be more representative of naturalistic puffing behavior, and results are consistent with the notion that a 30 sec IPI is typical of a tobacco cigarette smoker.

While standardized puffing protocols provide several benefits (e.g., allowing for comparison across products), they may not be representative of how individuals puff on non-cigarette tobacco products. That is, in one study of 29 experienced ECIG users, participants using their own device battery and ECIG liquid took significantly longer duration and larger volume puffs during 90 minutes of *ad libitum* puffing relative to a 10-puff directed bout (Spindle et al., 2017). For example, mean (SD) puff duration was 5.3 sec (2.1) during *ad libitum* puffing relative to 4.5 sec (1.6) during the directed bout (30 sec IPI). Similarly, mean (SD) puff volume was 148.5 ml (119.6) during *ad libitum* puffing and 124.6 ml (89.1) during the 10-puff directed bout (Spindle et al., 2017). Therefore, *ad libitum* protocols may capture more naturalistic behavior when assessing users of ECIGs (e.g., Farsalinos et al., 2014; Vansickel & Eissenberg, 2013) and other combustible tobacco products (Breland, 2005; Blank, 2009; Gust & Pickens, 1982; Herning et al., 1983; Rose & Behm, 2003). In an effort to obtain the benefits of both puffing protocols (directed and *ad libitum*), some studies of ECIGs employ both directed and *ad libitum* bouts (Spindle et al., 2015, 2017; Wagener et al., 2017). The review below includes several clinical laboratory studies in which ECIG associated nicotine delivery and subjective

effects have been assessed using either directed and/or *ad libitum* puffing protocols.

ECIG Nicotine Delivery. Nicotine delivery can be characterized as the amount of nicotine detected in the user's body and is often measured in venous blood plasma (i.e., plasma nicotine concentration, in ng/ml) following tobacco product use. Examining nicotine delivery from tobacco products, such as ECIGs, is important as the amount of nicotine and rate at which nicotine is delivered to the bloodstream helps to determine addiction potential (Henningfield & Keenan, 1993). When inhaled, nicotine from a combustible cigarette can reach the brain in approximately 10 to 20 seconds (Benowitz, 2008) and the rapidity with which nicotine from an ECIG reaches the bloodstream highlights the importance of measuring venous blood nicotine concentration immediately after use (Rüther, Hagedorn, Schiela, Schettgen, Osiander-Fuchs, & Schober, 2017).

To date, several clinical laboratory studies have been conducted to characterize the nicotine delivery from ECIGs (e.g., Dawkins & Corcoran, 2014; Dawkins et al., 2016, 2018; Farsalinos et al., 2014, 2015; Hiler et al., 2017; Nides, Leischow, Bhatter, & Simmons, 2014; Rüther, Hagedorn, Schiela, Schettgen, Osiander-Fuchs, & Schober, 2017; Spindle et al., 2015, 2017, 2018; Vansickel et al., 2010; St. Helen, Havel, Dempsey, Jacob, & Benowitz, 2015; Vansickel & Eissenberg, 2013; Yan & D'Ruiz, 2015). Importantly, across these studies, ECIG nicotine delivery varies widely, with data from some studies demonstrating ECIGs delivering little to no nicotine, cigarette-like levels of nicotine, or exceeding the nicotine delivery profile of tobacco cigarettes (i.e., ~15-20 ng/ml; Patterson et al., 2003; Vansickel et al., 2010; Yan & D'Ruiz, 2015). The review below will describe factors that contribute to the variability in ECIG nicotine delivery: device characteristics, liquid constituents and user experience.

Design Features: The nicotine delivery of an ECIG varies across different types of ECIG

models. In one within-subject study, differences in nicotine delivery were examined across a "cigalike" ("V2" ECIG with a cartomizer, W not reported) and a tank-based device ("EVIC" with "EVOD" atomizer, 9 W) in 23 experienced ECIG users (Farsalinos et al., 2014). ECIGs were filled with the same ECIG liquid ("Flavourart Maxblend," 18 mg/ml nicotine concentration, 35/65 PG/VG ratio) and participants completed one 5-minute, 10-puff directed bout (30 sec IPI) followed by a 60-minute *ad libitum* bout. Blood was sampled immediately following 10 puffs and every 15 minutes during the *ad libitum* bout for later measurement of plasma nicotine. Overall, the tank-based "EVIC" model, delivered significantly more nicotine to the user relative to the "cigalike" V2 model following 10 puffs and during the *ad libitum* bout (Farsalinos et al., 2014). That is, after 10 puffs, mean (SD) plasma nicotine concentration was 4.9 ng/ml (2.4) in the "cigalike" V2 condition and was 6.6 ng/ml (2.9) in the tank-based "EVIC" condition. At the conclusion of the *ad libitum* bout, mean (SD) plasma nicotine concentration was 15.8 ng/ml (5.8) in the "cigalike" condition compared to 23.5 ng/ml (9.1) in the tank-based "EVIC" condition (Farsalinos et al., 2014).

Results from a similar study demonstrated that experienced ECIG users (N = 9) obtain significantly more nicotine following 11 puffs from a tank-based "eGo-c" battery (attached to an "Aspire" atomizer; 1.8 Ω; W and V not reported) filled with 18 mg/ml nicotine concentration relative to three different types of "cigalikes" (i.e., American Heritage, Vype, and Blu; Rüther et al., 2017). In this study, plasma nicotine concentrations for the three "cigalike" conditions were averaged into a single value. Following 11 puffs, mean (SD) plasma nicotine concentration was 5.4 ng/ml (2.4) when using the "cigalike" devices and 9.2 ng/ml (5.4) when using the tank-based "eGo-c" (Rüther et al., 2017). Although more work is needed, the variability in nicotine delivery across different ECIG devices likely is a result of differences in device power output, as

suggested by ECIG aerosol studies (Talih et al., 2015). As will be described in detail later, ECIG devices with higher power result in increased nicotine delivery (Wagener et al., 2017).

Liquid constituents: Results from other ECIG studies demonstrate that liquid constituents (e.g., liquid nicotine concentration and the ratio of PG to VG) can influence nicotine delivery from an ECIG (Dawkins et al., 2016, 2018; Hiler et al., 2017; Spindle, 2017). In one two-condition study, 11 ECIG-experienced men were asked to use an "eVic supreme" (3.9 V; 8.5 W) ECIG attached to a "Nautilus Aspire" tank filled with either low (6 mg/ml) or high (24 mg/ml) liquid nicotine in two separate study sessions that consisted of a 60-minute *ad libitum* bout (Dawkins et al., 2016). Following ECIG use, plasma nicotine boost (i.e., increase in plasma nicotine concentration from baseline) was significantly higher in the 24 mg/ml condition relative to the 6 mg/ml condition at every measurement time point. For example, following 10 minutes of puffing, mean (SD) plasma nicotine boost was 8.6 ng/ml (7.5) in the 6 mg/ml condition and 33.8 ng/ml (34.9) in the 24 mg/ml condition. Following 30 minutes of puffing, mean (SD) plasma nicotine boost was 16.9 ng/ml (11.7) in the 6 mg/ml condition and 35.5 ng/ml (28.3) in the 24 mg/ml condition. Finally, after 60 minutes of puffing mean (SD) plasma nicotine boost was 22.0 ng/ml (16.2) in the 6 mg/ml condition and 43.6 ng/ml (34.8) in the 24 mg/ml condition. These results were observed despite the fact that puffing behaviors known to influence nicotine delivery (i.e., puff number and puff duration) were significantly higher in the 6 mg/ml condition relative to the 24 mg/ml condition. That is, in the 6 mg/ml condition, mean (SD) puff number was 70.7 (34.5) and puff duration was 5.2 sec (1.4) and in the 24 mg/ml condition puff number was 48.4 (22.9) and puff duration was 3.8 sec (1.0). Despite not controlling for participant puffing behavior, results from this study suggest that higher liquid nicotine concentration may lead to higher plasma nicotine concentrations. Another study demonstrated a direct relationship

between liquid nicotine concentration and plasma nicotine concentration in 33 experienced ECIG users who completed four laboratory sessions that consisted of two, 10-puff bouts (30 sec IPI; Hiler et al., 2017). In each session participants used an "eGo" ECIG battery (3.3 V; 7.3W) attached to a 1.5 Ω dual coil "cartomizer" filled with 1 ml of ECIG liquid (PG:VG ratio: 70:30). The four sessions differed only by the liquid nicotine concentration in the ECIG: 0, 8, 18, or 36 mg/ml. Results revealed that ECIG-associated nicotine delivery is related directly to liquid nicotine concentration. For example, immediately after 10 puffs, mean (SD) plasma nicotine boost was 0.01 ng/ml (1.5) in the 0 mg/ml condition, 8.2 ng/ml (7.8) in the 8 mg/ml condition, 13.0 ng/ml (6.2) in the 18 mg/ml condition, and 17.9 ng/ml (17.2) in the 36 mg/ml condition. Therefore, one important factor that explains user plasma nicotine concentration is liquid nicotine concentration.

Aside from liquid nicotine concentration, other ECIG liquid constituents such as ratio of PG to VG may influence ECIG nicotine delivery. In one within-subject study, 30 experienced ECIG users completed four sessions where they used an "eGo" ECIG (3.3 V; 7.3W) attached to a 510 dual-coil "cartomizer" filled with 18 mg/ml nicotine liquid (tobacco flavor; Spindle, 2018). Each session consisted of two separate 10-puff bouts (30 sec IPI) and sessions differed only by the PG:VG ratio in the ECIG liquid (100:0, 55:45, 20:80, and 2:98). Results revealed no significant differences across PG:VG ratio conditions immediately after the first bout. However, immediately after the second bout, plasma nicotine concentration was significantly higher in the 100 PG condition relative to the 20PG:80VG and 2PG:98VG conditions, and not the 55PG:45VG condition. That is, mean (SD) plasma nicotine concentration was 13.40 ng/ml (8.99) in the 100 PG condition, 9.59 ng/ml (7.95) in the 20PG:80VG condition, and 8.58 ng/ml (5.41)

in the 2PG:98VG condition (Spindle, 2017). These data suggest that other liquid constituents, aside from liquid nicotine concentration may be important predictors of nicotine delivery.

User experience: User experience with ECIGs is another factor that influences ECIG-associated nicotine delivery. All other factors being equal (e.g., device and liquid characteristics), experienced ECIG users are capable of obtaining more nicotine from these devices relative to ECIG-naïve cigarette smokers (Farsalinos et al., 2015; Hiler et al., 2017). In one single-condition study, nicotine delivery following ECIG use was compared across 24 experienced ECIG users and 23 ECIG-naïve cigarette smokers while device features and liquid nicotine concentration were held constant (Farsalinos et al., 2015). Participants who were ~8 hour abstinent completed a 10-puff directed bout and a 60-minute *ad libitum* bout using an "EVIC" ECIG battery (9 W) attached to an "EVOD" "tank" filled with 2 ml of 18 mg/ml nicotine concentration liquid ("Flavourart Maxblend," 35:65 PG:VG ratio; Farsalinos et al., 2015). Overall, experienced ECIG users had significantly greater blood plasma nicotine concentrations immediately after the 10-puff directed bout, and 15, 30, 45, and 60 minutes after the onset of the *ad libitum* bout (Farsalinos et al., 2015). Immediately after the 10-puff bout, mean (SD) plasma nicotine concentration for experienced ECIG users was 7.9 ng/ml (4.4) compared to 4.3 ng/ml (3.4) for ECIG-naïve smokers. Immediately after the 60-minute *ad libitum* bout mean (SD) plasma nicotine concentration for experienced ECIG users was 24.1 ng/ml (9.8) compared to 13.8 ng/ml (7.7) for ECIG-naïve smokers. Similarly, another study was conducted to evaluate ECIG-associated nicotine delivery across 33 experienced ECIG users and 31 ECIG-naïve cigarette smokers (Hiler et al., 2017). In this within- and between-subject study, all participants (ECIG-experienced and ECIG-naïve) underwent two 10-puff bouts that differed only by liquid nicotine concentration: 0, 8, 18, and 36 mg/ml. Nicotine delivery differed

significantly across experienced ECIG users and ECIG-naïve cigarette smokers, despite controlling for several characteristics that may influence nicotine delivery (e.g., device power, liquid PG:VG ratio, puff number). For example, when using 36 mg/ml liquid nicotine concentration, mean (SD) plasma nicotine boost for experienced ECIG users was 17.9 ng/ml (17.2) compared to 6.9 ng/ml (7.1) for ECIG-naive cigarette smokers. A similar pattern was observed for the other conditions in the study (i.e., 8 and 18 mg/ml), with the exception of the 0 mg/ml nicotine concentration. Given that both of the aforementioned studies compared nicotine delivery across experienced and naïve users, while holding other factors constant (i.e., device and liquid characteristics), the differential nicotine delivery across users is likely due to differences in user puffing behavior. Differences in puffing behavior between experienced ECIG users and those who are inexperienced with ECIGs will be described in detail below.

Subjective Effects. Because nicotine is a psychoactive ingredient of ECIG liquid and other tobacco products, its delivery to the blood can influence user subjective experience. Therefore, in addition to delivering nicotine to the user, ECIGs may produce subjective effect profiles capable of producing and maintaining nicotine dependence by alleviating nicotine/tobacco abstinence symptoms (i.e., craving, depressed mood, irritability, anxiety, frustration, and difficulty concentrating) and producing pleasurable effects (e.g., mild euphoria, increased energy, and heightened arousal) following use (Eissenberg, 2004; Glautier, 2004; Nides et al., 2014; Spindle et al., 2018). As such, in addition to assessing nicotine/tobacco abstinence symptoms of ECIG users, incorporating measures to examine “satisfaction”, “pleasantness”, “liking” as well as other sensory effects (i.e., sight, smell, taste, and mouth feel) is important given these measures are sensitive and valid markers of drug abuse potential (Carter et al., 2009). Numerous clinical laboratory studies have been conducted to evaluate ECIG-

induced abstinence symptom suppression and other subjective effects associated with ECIG use among ECIG-naïve cigarette smokers and experienced ECIG users (Dawkins & Corcoran, 2014; Dawkins et al., 2016; Farsalinos et al., 2014, 2017; Hiler et al., 2017; Nides et al., 2014; Rüther et al., 2017; Spindle et al., 2015, 2017, 2018; Vansickel et al., 2010; Vansickel & Eissenberg, 2013; Wagener et al., 2017). Importantly, several of the factors that influence nicotine delivery (device characteristics, liquid constituents, and user experience) also influence ECIG subjective effects.

Device characteristics: As seen with nicotine delivery, ECIG device characteristics may influence ECIG-induced abstinence symptom suppression and other subjective effects (Dawkins, Kimber, Puwanesarasa, & Soar, 2015; Farsalinos et al., 2014; Lechner, Meier, Wiener, Grant, & Gilmore, 2015). In one study, 97 cigarette smokers were assigned randomly to undergo a 10 puff bout (3 sec puff duration with 30 sec IPI) from either a "cigalike" device from "Totally Wicked" with 18 mg/ml nicotine concentration or a tank-based "eGo-T" ECIG filled with 18 mg/ml tobacco flavored liquid (voltage, wattage not reported for either device; Dawkins et al., 2015). Nicotine/tobacco abstinence symptoms were assessed before and after the 10 puffs using the Mood and Physical Symptoms Scale that includes items such as "urge to smoke". Although both devices reduced "urge to smoke", participants using the tank-based "eGo-T" reported significantly more satisfaction with the device. However, because nicotine delivery was not an outcome of this study, the extent to which differences in nicotine delivery across the two devices can explain the observed differences in satisfaction ratings is unclear (Dawkins et al., 2015). Another study revealed that experienced ECIG users report significantly lower levels of craving and more pleasurable effects after using a tank-based ECIG relative to a "cigalike" (Farsalinos et al., 2014). In a within-subject study design, 23 experienced ECIG users completed two sessions

that included a 10-puff ECIG use bout (30 sec IPI) from either a "cigalike" ("V2"; W not reported) or a tank-based ECIG ("EVIC" with "EVOD" atomizer; 9 W), while ECIG liquid ("Flavourart Maxblend," 18 mg/ml) was held constant (Farsalinos et al., 2014). Scores for the subjective item "craving" were significantly lower following use of the tank-based "EVIC" relative to the "cigalike" V2. Moreover, scores for items such as "satisfying" and "throat hit" were significantly higher following 10 puffs from the tank-based "EVIC" relative to the "cigalike". Further, these observed differences in subjective effects across the two devices can be explained by differences in nicotine delivery as the tank-based "EVIC" delivered significantly more nicotine to the user (Farsalinos et al., 2014).

Liquid constituents: Constituents of ECIG liquid also influence user subjective experience. That is, relative to ECIG liquids that contain no nicotine, those that do contain nicotine suppress nicotine/tobacco abstinence symptoms more effectively (Dawkins, Turner, & Crowe, 2013; Dawkins, Turner, Hasna, & Soar, 2012; Hiler et al., 2017). In one within-subject study, 20 cigarette smokers underwent two laboratory sessions, in which they used a "Tornado" cartridge-based ECIG filled with "Totally Wicked" ECIG liquid. Sessions differed only by the liquid nicotine concentration in the ECIG: either 0 mg/ml (placebo) or 18 mg/ml liquid nicotine concentration (Dawkins et al., 2013). In each session, ~8-10 hour abstinent participants used the "Tornado" ECIG *ad libitum* for 10 minutes and nicotine/tobacco abstinence symptoms were assessed before and after product administration using the desire to smoke scale and the Mood Physical Symptoms Scale (MPSS; e.g., depressed mood, irritability, restlessness, anxiety, drowsy, difficulty concentrating, and hunger). Overall, the desire to smoke and abstinence symptom scores on the MPSS were significantly lower after using the ECIG containing nicotine (18 mg/ml) relative to non-nicotine containing ECIG (0 mg/ml nicotine concentration; Dawkins

et al., 2013).

Indeed, high concentration nicotine liquids reduce abstinence symptoms to a greater magnitude relative to low concentration nicotine liquids (Dawkins et al., 2016, 2018; Hiler et al., 2017). One within-subject study varied liquid nicotine concentration, held device features constant, and evaluated subjective effects in experienced ECIG users during *ad libitum* puffing (Dawkins et al., 2016). In this study, 11 ECIG-experienced men used an "eVic supreme" ECIG attached to a "Nautilus Aspire" tank (3.9 V; 8.5 W) filled with either low (6 mg/ml) or high (24 mg/ml) liquid nicotine in two separate study sessions that consisted of 60 minutes of *ad libitum* puffing. Nicotine/tobacco abstinence symptom suppression and pleasurable effects such as "throat hit" and "satisfaction" were measured. Overall, while nicotine/ tobacco abstinence symptoms decreased significantly from baseline, scores did not differ significantly across conditions (6 vs. 24 mg/ml). Scores for "hit" and "satisfaction" demonstrated trends towards significance ($p = 0.09$). For example, mean (SD) satisfaction ratings were 60.7 (17.30) in the 24 mg/ml condition and 46.9 (16.9) in the 6 mg/ml condition. The lack of statistical significance across conditions in this study may be a result of lack of experimental control as puff number varied significantly across conditions (e.g., 70.7 puffs in the 6 mg/ml condition and 48.4 puffs in the 24 mg/ml condition) and a lack of statistical power due to limited sample size (N = 11).

Another study varied liquid nicotine concentration and examined nicotine/tobacco abstinence symptom suppression in experienced ECIG users using a controlled puffing regimen (Hiler et al., 2017). In this study, 33 experienced ECIG users completed four laboratory conditions that differed only by liquid nicotine concentration: 0, 8, 18, and 36 mg/ml. In each session, participants used an "eGo" ECIG battery (3.7 V; 7.3 W; 1.5 Ω) during two 10-puff directed bouts. Relative to baseline, significant reductions in nicotine/tobacco abstinence

symptoms were observed for the modified Hughes-Hatsukami items “craving”, “depression”, “drowsy”, and “urges to use an e-cigarette” and for Questionnaire of Vaping Craving Factors 1 and 2. Notably, decreases in abstinence symptom suppression were more pronounced when higher liquid nicotine concentrations were used. For example, for the Hughes-Hatsukami item “urge”, mean (SD) scores immediately after the first bout were 54.1 (34.6) in the 0 mg/ml condition, 40.5 (30.0) in the 8 mg/ml condition, 34.8 (25.8) in the 18 mg/ml condition, and 26.6 (25.8) in the 36 mg/ml condition. Similarly, other subjective effects such as “pleasant” and “satisfy” were significantly higher in each active nicotine condition (i.e., 8, 18, and 36 mg/ml) relative to the 0 mg/ml condition. Immediately after the first bout, mean (SD) scores for “satisfy” were 33.4 (33.1) in the 0 mg/ml condition, 50.8 (33.0) in the 8 mg/ml condition, 61.0 (30.5) in the 18 mg/ml condition, and 60.0 (30.1) in the 36 mg/ml condition. Collectively, these studies suggest that higher concentration nicotine liquids reduce abstinence symptoms and produce more pleasurable subjective effects (e.g., “satisfy”) to a greater degree than lower concentration nicotine liquids.

ECIG subjective effects are also influenced by flavors present in ECIG liquid (Krishnan-Sarin, Green, Kong, Cavallo, Jatlow, Gueorguieva, Buta, and O’Malley, 2017; Rosbrook & Green, 2016). The effects of flavors and nicotine were assessed in a study of 60 young adult ECIG users, who completed three sessions using a tank-based “V2 Cigs” ECIG (V and W not reported) filled with one of three different concentrations of nicotine (0, 6, and 12 mg/ml; Krishnan-Sarin, 2017). During each of the three study sessions, participants used an ECIG with their assigned nicotine concentration and one of three varying levels of menthol flavoring: 0% (no menthol), 0.5% (low menthol), 3.5% (high menthol; Krishnan-Sarin et al., 2017). Each session consisted of a 30-minute “fixed dose” period (consisting of three, 10-puff bouts separated

by 10 minutes) followed by 30 minutes of *ad libitum* puffing. Subjective measures included the general Labeled Magnitude Scale (gLMS) to assess the overall intensity/coolness of the ECIG and the labeled Hedonic Scale (LHS) to assess liking/disliking of the ECIG taste. Overall, the presence of high concentration of menthol in the liquid significantly increased ECIG liking/wanting relative to no menthol, regardless of the liquid nicotine concentration. Also, relative to the no menthol condition the low and high menthol conditions resulted in significant improvements in the taste of the ECIG and cooling sensations associated with ECIG use (Krishnan-Sarin et al., 2017). A similar study evaluated the interactive effects of menthol and nicotine in 32 adult cigarette smokers who sampled a "cigalike" ECIG ("V2") with five different concentrations of nicotine (0, 6, 12, 18, and 24 mg/ml) with varying levels of menthol flavor (0%, 0.5%, 3.5%; Rosbrook & Green, 2016). Following ECIG use, gLMS scores revealed that the presence of menthol (i.e., 0.5%, 3.5%) in ECIG liquid significantly reduced perceived airway irritation/harshness when paired with the 24 mg/ml nicotine concentration (Rosbrook & Green, 2016). These results suggest that ECIG liquids containing menthol flavors may impact ECIG-induced subjective experience by altering the perception of harshness of nicotine and by increasing the overall liking/wanting of the product.

The effects of other ECIG liquid flavors compared to unflavored liquids have also been evaluated in the laboratory. In a within-subject study of 32 adult cigarette smokers, participants completed single study session where they sampled an "eGo" tank-based ECIG filled with nicotine-containing liquid in fruit flavor (apple), dessert flavor (chocolate), or no flavor (Audrain-McGovern, Strasser, & Wileyto, 2016). Each of the ECIG samplings were separated by 20 minutes, and subjective ratings of "satisfaction" and "good taste" were averaged to produce a single value of "subjective reward". Overall, participant subjective reward ratings were

significantly higher for the flavored ECIGs (fruit and dessert) relative to the unflavored. Furthermore, subjective reward ratings were significantly higher for fruit relative to dessert flavor (Audrain-McGovern et al., 2016). In sum, the presence of flavorants in ECIG liquids is an important factor in increasing participant subjective ratings of ECIG use.

The ratio of PG and VG present in ECIG liquids is another factor that influences subjective effects following ECIG use (Harvanko et al., 2019; Spindle, 2018). In a within-subject study of 30 experienced ECIG users, participants completed four sessions consisting of two, 10-puff directed bouts (30 sec IPI) where they used an "eGo" ECIG with a dual-coil cartomizer (3.3V; 7.3W; 1.5 Ω) filled with 18 mg/ml ECIG liquid that differed only by the PG:VG ratio of the liquid: 2:98, 20:80, 55:45, 100:0 (Spindle, 2018). Overall, nicotine/tobacco abstinence symptom suppression (e.g., "urge", "craving", "irritable") did not differ across conditions although significant differences for other subjective effects (i.e., "satisfaction", "pleasant", "throat hit") were observed. For example, scores for the Direct Effects of ECIG use scale item "satisfy" were significantly lower in the 100 PG condition relative to other conditions. Following 10 puffs, mean (SD) scores for "satisfy" were 52.60 (30.98) in the 100 PG condition and 67.70 (25.83) in the 2PG:98VG condition, suggesting higher satisfaction when using the predominantly VG liquid. Similar patterns were observed for the items "pleasant", "calm", and "awake". Also, scores for the gLMS item "throat hit" were significantly higher for the 100 PG condition relative to the 2 PG:98 VG and 20 PG:80 VG conditions immediately after the first bout. These results suggest that PG:VG ratio does not influence reduction in nicotine/tobacco abstinence symptom ratings, but may influence satisfaction and other sensory effects associated with ECIG use. Overall, ECIG liquid constituents such as liquid nicotine concentration, flavorants, and PG:VG ratio influence subjective ratings following ECIG use to some capacity.

User experience: Several studies have examined ECIG-induced subjective effects in ECIG-naïve cigarette smokers (Dawkins et al., 2012; Dawkins et al., 2013; Nides et al., 2014; Vansickel et al., 2010) and experienced ECIG users independently (Dawkins & Corcoran, 2014; Krishnan-Sarin et al., 2017; Rosbrook & Green, 2016; Spindle et al., 2018; Vansickel & Eissenberg, 2013). To date, only one study examined the extent to which user experience influences subjective effects following ECIG use (Hiler et al., 2017). In this within and between-subject study, 33 experienced ECIG users and 31 ECIG-naïve cigarette smokers completed four study conditions consisting of two, 10-puff directed ECIG use bouts that differed only by ECIG liquid nicotine concentration (i.e., 0, 8, 18, or 36 mg/ml). Although ECIG use suppressed nicotine/tobacco abstinence symptoms for both groups, experienced ECIG users reported greater abstinence symptom suppression relative to ECIG-naïve cigarette smokers. For example, experienced ECIG users reported significantly lower scores for the Hughes-Hatsukami item "urge" after the first 10 puffs of the ECIG in the 18 mg/ml condition: mean (SD) "urge" score was 34.8 (26.0) for experienced ECIG users and 56.3 (34.5) for ECIG-naïve cigarette smokers. Similar patterns were observed for other items such as "craving" and QSU-brief Factors 1 and 2 (i.e., intention to smoke and anticipation of relief from withdrawal symptoms). Overall, data from this study indicates that ECIG use suppresses nicotine/tobacco abstinence symptoms for both groups; however, the magnitude of abstinence symptom suppression depends, in part, on user experience.

Collectively, the results from several clinical laboratory studies have revealed substantial variability in nicotine delivery and subjective experience across devices, liquid constituents, and user experience. Another factor that also influences nicotine delivery is user behavior (i.e., puff topography). Measurement of puff topography in cigarettes and ECIGs (as described below) is

relevant to understanding the nicotine and toxicant exposure of inhalable tobacco products and therefore is an important component of studies designed to inform ECIG regulation.

Puff Topography

Puff topography measurement involves assessment of puffing behaviors such as puff number, puff duration, puff volume, IPI, and flow rate (i.e., puff velocity) during tobacco product use (Blank, Disharoon, & Eissenberg, 2009). Previously, puff topography assessment helped to explain the relationship between nicotine intake and exposure to other harmful tobacco constituents following combustible cigarette use (Gust & Pickens, 1982; Herning, Jones, Benowitz, & Mines, 1983). Puff topography measurement through observational methods or through use of mouthpiece-based computerized devices has been used to evaluate and characterize puffing behavior during ECIG-use. The review below will highlight the importance of measuring puff topography in toxicant exposure of tobacco products, describe two methods for assessing puff topography, and explain how these methods are applied to assess the puffing behaviors of ECIG users.

Importance of Puff Topography in Toxicant Exposure: Early measurement of puff topography in tobacco cigarette smokers revealed that individual exposure to nicotine and other toxicants is determined largely by puffing behavior (Herning, Jones, Benowitz, & Mines, 1983; Sutton, Russell, Iyer, Feyerabend, & Saloojee, 1982; Zacny, Stitzer, Brown, Yingling, & Griffiths, 1987). For example, in a repeated measures experimental study of 10 cigarette smokers, participants varied puff volume across sessions (15, 30, 45, and 60 ml) while holding constant their breathhold duration (4 sec) and puff number (8 puffs; Zacny et al., 1987). Results revealed that nicotine and CO boost (i.e., increase in nicotine and CO from baseline) increased as puff volume increased. That is, mean nicotine boost was 4.6 ng/ml with 15 ml puff volume, 7.5

ng/ml with 30 ml puff volume, 11.85 ng/ml with 45 ml puff volume, and 16.25 ng/ml with 60 ml puff volume. Similarly, mean CO boost was 1.0 ppm with 15 ml puff volume, 4.2 ppm with 30 ml puff volume, 6.3 ppm with 45 ml puff volume, and 8.7 ppm with 60 ml puff volume. These results demonstrate that cigarette smoker puffing behavior has a direct influence on nicotine and CO exposure, emphasizing the importance of measuring puff topography during tobacco use episodes (Zacny et al., 1987).

Measurement of puff topography has also been essential to understanding toxicant exposure of novel tobacco products such as "low-yield" cigarettes. In the 1980's, "low-yield" cigarettes were marketed with claims of reducing smoking-related harm by reducing exposure to toxicants such as "tar", defined as nicotine-free, dry particulate matter (Davis, 1987). These claims were reinforced by the Federal Trade Commissions (FTC) characterization of cigarettes as "full-flavor", "light" and "ultra-light" based on results from machine-smoked cigarette yields (i.e., smoke constituent concentrations). Machine-based smoking was used by the FTC to measure smoke related constituents produced by burning cigarettes according to a standard puffing procedure (i.e., 2 sec puff duration, 35 ml puffs performed every 58 sec until the cigarette reaches a length of 23 mm). According to machine-based smoking of "low-yield" cigarettes, these cigarettes did indeed yield lower concentrations of nicotine, CO, and "tar" relative to "full flavor" cigarettes (Hoffmann, Djordjevic, & Hoffman, 1997; FTC, 2000). However, clinical laboratory studies revealed that "low-yield" cigarettes exposed users to toxicant levels comparable to that of "full flavor" cigarettes (Baldinger, Hasenfratz, & Battig, 1995; Benowitz et al., 1983; Gust & Pickens, 1982; Herning, Jones, Bachman, & Mines, 1981; Zacny & Stitzer, 1988) perhaps as a result of more intensive puffing when switching from "full-flavor" to "low-yield" cigarettes (Benowitz, et al., 2005; Gust & Pickens, 1982; Zacny & Stitzer,

1988).

According to results from one laboratory study, when 24 overnight abstinent cigarette smokers used either "low," "medium," or "full flavor" cigarettes (while holding tar, and CO yield constant), participants took significantly larger volume puffs when smoking "low-yield" cigarettes (Herning et al., 1981). Also, after smoking "low-yield" cigarettes the resulting expired air CO was consistent with that of smoking a "full flavor" cigarette, indicating that "low-yield" cigarettes would do little to reduce smoking-related harm. Other studies have found similar changes in puffing behavior when switching from "full-flavor" to "low-yield" cigarettes, and these changes in puff topography are often accompanied by toxicant exposure that is similar across the two products (Gust & Pickens, 1982; Zacny & Stitzer, 1988). Results from the aforementioned examinations and others (e.g., Herning et al., 1983; Kassel et al., 2007; Strasser, Lerman, Sanborn, Pickworth, & Feldman, 2007; Sutton et al., 1982; Zacny & Stitzer, 1988) highlight the importance of measuring puff topography during tobacco use in order to understand user exposure to nicotine and other toxicants.

Measurement of Puff Topography. Two methods often used for puff topography measurement are direct observation or mouthpiece-based topography devices. Observational methods oftentimes include the use of a video camera to record participant behavior in the laboratory (Blank, 2008; Blank et al., 2016; Frederiksen, Miller, & Peterson, 1997; Lichtenstein & Antonuccio, 1981). Subsequently, the video recordings are scored by trained observers who determine puff number by counting the number of puffs taken during a session and puff duration by measuring the time between puff onset and offset (Blank et al., 2009, Blank et al., 2016). Observational methods to assess puff topography are reliable and valid, although the video scoring process is time consuming and labor intensive. Moreover, observational methods are

unable to measure puff volume and flow rate (Blank et al., 2009; Blank et al., 2016). To overcome the concerns associated with observational methods, mouthpiece-based computerized devices are often used to measure puff topography.

Measurement of puff topography through the use of mouthpiece-based computerized devices requires placement of a tobacco product (e.g., cigarette or ECIG) into a specialized mouthpiece that is capable of detecting flow-induced pressure changes that occur as a result of user inhalation. A pressure transducer and associated pre-calibrated software converts pressure changes to flow rate and uses this information to calculate puff duration, volume, and IPI (Blank, 2008). One mouthpiece-based topography device, known as the Clinical Research Support System (CReSS), has been validated and used in laboratory studies to measure cigarette smoker puff topography (Blank et al., 2009; Buchhalter & Eissenberg, 2000). Although the device was created to measure smoking behavior, the CReSSMicro has also been used extensively to measure ECIG-use topography (Behar, Hua, Talbot, 2015; Goniewicz, et al., 2013; Lee, Gawron, & Goniewicz, 2015; Norton, June, O'Connor, 2014). However, devices such as CReSS, that were developed for examining cigarette smoking behavior, face measurement challenges when used with ECIGs (Blank et al., 2016). For example, existing mouthpiece-based devices have standardized mouthpieces intended to fit cigarettes, thereby limiting the types of ECIGs for which topography can be measured (Blank et al., 2016). Because the ECIG must fit inside the mouthpiece firmly, only certain ECIGs that resemble cigarettes (i.e., cigalikes) can be evaluated. Moreover, topography devices intended for cigarette smokers may not be sensitive enough to detect the low flow rate puffs (i.e., 15 ml/sec or below) that have been observed among some experienced ECIG users (Behar, Hua, & Talbot, 2015; Spindle et al., 2015). Perhaps in response to concerns over size restrictions and flow rate sensitivity, several mouthpiece-based

computerized topography recording devices have been designed specifically for use with ECIGs (Spindle et al., 2015, 2017; Robinson, Hensel, Morabito, & Roundtree, 2015). One such device was developed by researchers at the American University of Beirut (AUB) and is intended to operate similarly to devices used to measure cigarette smoker's puff topography (e.g., CReSS) while also providing the sensitivity to capture low flow rate puffs accurately (Spindle et al., 2015, 2017). Studies that have evaluated puff topography during ECIG use using observation and mouthpiece-based methods are described below.

ECIG Puff Topography Measurement. Several studies have used observational methods to assess ECIG user puff topography (Farsalinos, Romangna, Tsiapras, Kyrzopoulos, & Voudris, 2013; Hua, Yip, & Talbot, 2013; St. Helen et al., 2016; St. Helen, Shahid, Chu, Benowitz, 2017). For example, in one observational study in which YouTube videos of 64 ECIG users and 9 cigarette smokers were assessed, ECIG users took significantly longer duration puffs relative to cigarette smokers (Hua et al., 2013). Mean (SD) puff duration observed for ECIG users was 4.3 sec (1.5) and for cigarette smokers observed puff duration was 2.4 sec (0.8; Hua et al., 2013). Another observational study revealed similar differences in puffing behavior when 45 experienced ECIG users and 35 ECIG-naïve tobacco cigarette smokers were asked to use an "eGo-T" tank-based ECIG (9 mg/ml liquid nicotine concentration) *ad libitum* in the laboratory (Farsalinos et al., 2013). Experienced ECIG users underwent a 20-minute *ad libitum* bout while ECIG-naive cigarette smokers completed two 10-minute *ad libitum* bouts, one with the "eGo-T" and the other with their own brand cigarette. Experienced ECIG users took significantly longer puffs when using the "eGo-T" relative to ECIG-naïve smokers when using the "eGo-T" or their own brand cigarettes. For example, mean (SD) puff duration for experienced ECIG users was 4.2 sec (0.7) and for ECIG-naïve cigarette smokers puff duration was 2.4 sec (0.5) when using the

"eGo-T" ECIG and 2.1 sec (0.4) when using their own brand cigarette (Farsalinos et al., 2013). Similar differences in puff topography across experienced ECIG users and ECIG-naïve cigarette smokers have been reported in studies using mouthpiece-based computerized topography devices specifically designed for ECIGs (Spindle et al., 2015, 2017, 2018; Hiler et al., 2017). For example, in a within-subject study, 29 experienced ECIG users used their preferred ECIG battery and liquid and an experimenter-provided cartomizer during two sessions that differed by the presence of the topography device (and its mouthpiece attachment). Each session included a 10-puff directed bout (30 sec IPI) and a 90-minute *ad libitum* bout and included measurement of plasma nicotine concentration and subjective effects. Overall, the presence of the topography recording device did not influence ECIG acute effects (i.e., plasma nicotine concentration and subjective effects). This study also compared puffing results (i.e., volume, duration, and flow rate) from 29 ECIG users to 123 tobacco cigarette smokers from a previous study completed in the same laboratory under similar conditions (Kleylamp, 2008). Experienced ECIG users, using their preferred battery and liquid, took longer and larger puffs as well as slower flow rate puffs relative to cigarette smokers, using their own brand cigarettes. For example, ECIG users took longer duration puffs lasting 4.5 sec (1.6) compared to 1.4 sec (0.4) for cigarette smokers. Similarly, ECIG users took larger volume puffs that were 124.6 ml (89.1) compared to 51.3 ml (19.2) for cigarette smokers. Finally, ECIG users flow rate was 27.8 ml/sec (19.5) compared to cigarette smokers flow rate of 38.0 ml/sec (9.7; Spindle et al., 2015, 2017). Overall, this study validated a mouthpiece-based topography recording device specific for use with ECIGs by demonstrating that the device did not interfere with ECIG acute effects (i.e., nicotine delivery, heart rate and subjective effects) and also demonstrated significant differences in puff topography across experienced ECIG users and cigarette smokers (Spindle et al., 2015, 2017).

Finally, one study to date used the above-mentioned puff topography device to evaluate differences in puff topography across experienced ECIG users and ECIG-naïve smokers under controlled conditions (i.e., holding device features constant). In this within and between-subject study, puff topography was measured in 33 experienced ECIG users and 31 ECIG-naïve cigarette smokers across four sessions that differed by liquid nicotine concentration (i.e., 0, 8, 18, or 36 mg/ml liquid nicotine concentration; Hiler et al., 2017). Collapsed across liquid nicotine concentration condition, mean (SD) puff duration was 5.6 sec (3.0) for experienced ECIG users and 2.9 sec (1.5) for ECIG-naïve smokers. Also, collapsed across condition, mean (SD) puff volume was 151.8 ml (134.6) for experienced ECIG users and 89.1 ml (63.7) for ECIG-naïve smokers. However, mean (SD) puff flow rate did not differ significantly across groups and was 27.9 ml/sec (19.2) for experienced ECIG users and 31.9 ml/sec (22.0) for ECIG-naïve smokers. In this study, the between group differences in puff topography suggest a relationship between longer/larger puffs and higher plasma nicotine concentration as experienced ECIG users obtained significantly greater mean plasma nicotine concentrations relative to ECIG-naïve cigarette smokers at each active liquid nicotine concentration. Also, puff topography was related to liquid nicotine concentration with longer/larger puffs being observed in the lower liquid nicotine concentration conditions. For example, during the first bout, collapsed across group, mean (SD) puff duration was 4.7 sec (2.4) in the 0 mg/ml condition, 4.4 sec (4.0) in the 8 mg/ml condition, 4.0 sec (3.7) in the 18 mg/ml condition, and 3.5 sec (2.8) in the 36 mg/ml condition. For puff volume, during the first bout, collapsed across group, mean (SD) puff volume was 140.1 ml/sec (121.8) in the 0 mg/ml condition, 142.5 ml/sec (116.7) in the 8 mg/ml condition, 107.4 ml/sec (73.5) in the 18 mg/ml condition, and 96.7 ml/sec (130.7) in the 36 mg/ml condition. Thus far, measurement of puff topography during ECIG use has helped explain differences in nicotine

delivery across experienced ECIG users and ECIG-naïve smokers. Also, measurement of ECIG puff topography has revealed that users take larger/longer puffs when using lower concentration nicotine liquids. Continued puff topography measurement will be important when evaluating ECIGs and other novel tobacco products to inform regulation.

Need for Systematic Evaluation of ECIG Device and Liquid Characteristics

The variability in ECIG device and liquid characteristics and their increased use prevalence among various populations (i.e., adults, adolescents, and nicotine-naïve individuals) is a growing public health concern. In an effort to protect public health, U.S. FDA intends to regulate ECIGs and their "parts and components" (Deeming, 2016). One particular concern regarding ECIGs is the extent to which these devices support nicotine/tobacco dependence. Understanding the dependence potential of ECIGs will necessitate an understanding of the factors that influence nicotine delivery (i.e., device characteristics, liquid constituents, and user puff topography) and produce subjective effect profiles consistent with maintaining or producing nicotine dependence. To enforce effective ECIG policies, regulators will require empirical evidence that addresses the extent to which specific ECIG device and liquid characteristics influence nicotine delivery from these products. Systematic evaluation of ECIGs can accomplish this goal by holding certain factors constant (e.g., device battery, voltage, PG:VG ratio, flavor) while manipulating others (e.g., device power via heating coil resistance and liquid nicotine concentration). With systematic evaluation, the manipulated variables could be considered to be influencing outcomes of interest. Despite the growing ECIG literature, no studies to date have evaluated systematically the individual and combined effects of ECIG coil resistance and liquid nicotine concentration on ECIG acute effects (i.e., nicotine delivery, subjective effects, heart rate) and user puff topography.

Why Examine ECIG Coil Resistance?

ECIGs vary markedly in terms of user-controlled design features (e.g., voltage and coil resistance) and liquid nicotine content (Breland et al., 2017; Etter, 2012). Device voltage, the force that drives an electrical current through the ECIG heating coil, is narrowly constrained (e.g., 3-6 V). Conversely, the resistance of the coil and the nicotine concentration of ECIG liquid can vary substantially (e.g., 0.1-6.5 Ω; 0-36 mg/ml; Breland et al., 2017; Wagener et al., 2017). With regards to coil resistance, the resistance of heating coils determines the ease with which electricity can travel through the device such that lower resistance coils allow for electricity to travel through the device with more ease.

Whereas conventional ECIG models operate with relatively high resistance coils (e.g., 1.5 Ω or higher), more recent ECIG models contain coils with a resistance below 1 Ω (Breland et al., 2017; Krielgel, 2015; Talih et al., 2017b; Wagener et al., 2017). The use of ECIGs with a resistance of <1 Ω is referred to colloquially as "sub-Ohm vaping" (Laestadius et al., 2016). Further, due to the inverse relationship between device power and coil resistance, "sub-Ohm" devices are capable of operating at higher power (e.g., 50-300 W) relative to conventional devices (Talih et al., 2017b). Indeed, a common method for increasing device power involves decreasing coil resistance. Due to the novelty of these lower-resistance heating coils, few studies have investigated the influence of ECIG coil resistance on nicotine yield or nicotine delivery. The review below will describe the limited data that are available currently regarding high power devices and those devices that contain low resistance coils.

The impact of power output on ECIG nicotine yield has been examined in a few pre-clinical laboratory studies (Kosmider et al., 2014; Talih et al., 2015) with data from one study demonstrating an approximate 4- to 5-fold increase in nicotine yield when device power was

increased from 3 to 7.5 W by increasing the voltage from 3.3 to 5.2 V (Talih et al., 2015). Importantly, in this study all other factors such as coil resistance (3.6 Ω), puff duration (4 sec), flow rate (17 ml/sec) and nicotine concentration (8.53 mg/ml) were held constant (Talih et al., 2015). Although this study used a device with a 3.6 Ω heating coil, device power is a function of both voltage and coil resistance (i.e., $W=V^2/\Omega$), and increasing device power by lowering coil resistance would likely increase nicotine yield.

To date, one clinical laboratory study demonstrated the importance of device power with <1 Ω coil resistance on ECIG-associated nicotine delivery when 20 experienced ECIG users (~12-hour nicotine abstinent) completed a single laboratory session consisting of a 10-puff directed bout (30 sec IPI) followed by a 115-min *ad libitum* bout with their preferred ECIG device and liquid (Wagener et al., 2017). Investigators categorized participants as either "second" or "third" generation ECIG users based on device power. That is, "second generation" ECIG users (N =9) reported mean (SD) device power of 8.6 W (1.9), voltage of 4.1 V (0.5), and coil resistance of 2.0 Ω (0.3). "Third generation" ECIG users (N = 11) self-reported mean (SD) device power of 71.6 W (50.0), voltage of 4.0 V (0.4), and coil resistance of 0.4 Ω (0.2). While device power and coil resistance differed significantly across "second" and "third" generation devices, voltage did not. Results indicated that following 10 puffs from their own device and liquid, "third generation" ECIG users (0.4 Ω; 71.6 W) obtained significantly more nicotine relative to "second generation" ECIG users (2.0 Ω; 8.6 W; see Figure 2). Mean (SD) plasma nicotine concentration was 7.3 ng/ml (2.8) for "second generation" ECIG users (2.0 Ω; 8.6 W) relative to plasma nicotine concentrations of 17.5 ng/ml (12.9) for "third generation" ECIG users (0.4 Ω; 71.6 W).

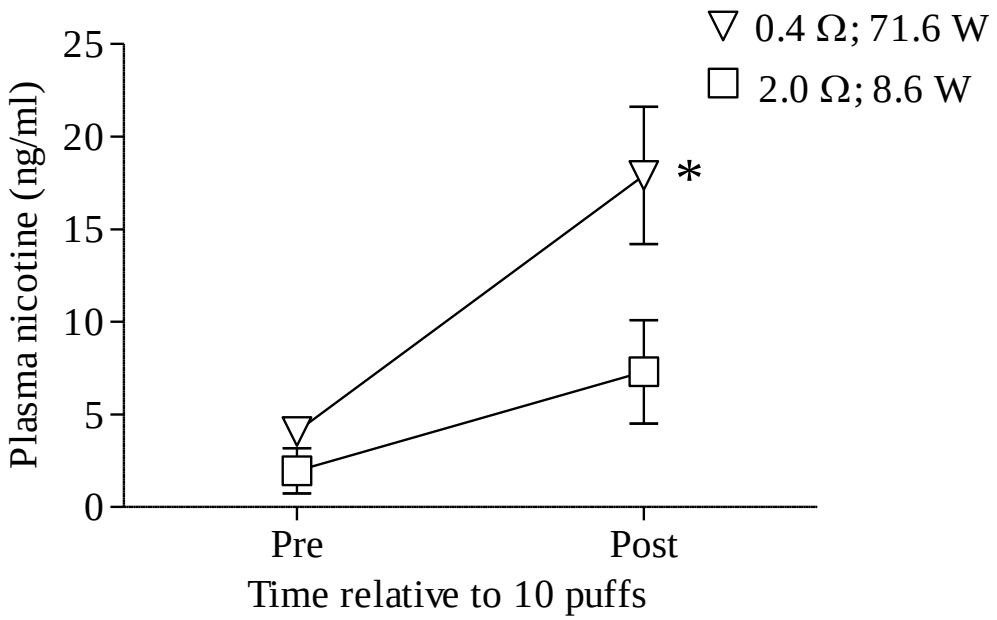

Figure 2. Mean (+/- SEM) plasma nicotine concentration following 10 puffs from 11 experienced ECIG users using devices with mean 0.4 Ω; 71.6 W and nine experienced ECIG users using devices with mean 2.0 Ω; 8.6 W. Mean liquid nicotine concentration was 4.1 mg/ml for the 0.4 Ω; 71.6 W devices and 22.3 mg/ml for the 2.0 Ω; 8.6W devices. Asterisk indicates significant between group difference for that time point ($p < .05$). Data for figure from Wagener et al., 2017.

Notably, “third generation” ECIGs (0.4 Ω; 71.6 W) delivered nicotine more effectively despite being paired with liquid nicotine concentrations that had a mean of 4.1 mg/ml; “second generation” devices (2.0 Ω; 8.6 W) were paired with liquid nicotine concentration with a mean of 22.3 mg/ml. The use of high power devices with low resistance coils may also increase ECIG liquid consumption. In the above-referenced study, users of “third generation” devices inhaled almost three times more liquid, by weight, relative to users of “second generation” devices. For example, users of “third generation” ECIGs (0.4 Ω; 71.6 W) consumed mean (SD) 4.7 mg (2.3) of liquid whereas “second generation” users (2.0 Ω; 8.6 W) consumed 0.5 mg (0.3; Wagener et al., 2017). In sum, high power devices with low resistance coils are effective at delivering nicotine to the user, even when paired with a low liquid nicotine concentration (i.e., 1.5-6 mg/ml) and they increase liquid consumption significantly (Wagener et al., 2017). However, the influence of coil resistance on nicotine delivery remains unclear because device power, coil resistance and liquid nicotine concentration varied widely across individuals (e.g., 18.6-162.4 W; 0.1-2.6 Ω; 1.5-36 mg/ml) and were not manipulated systematically. Furthermore, user puff topography was not measured and potential differences in user puffing behavior across “second” and “third generation” devices are unknown.

As with nicotine delivery, the influence of high power devices with low resistance coils on user subjective experience remains unclear. In the aforementioned examination of “second generation” and “third generation” ECIGs, participants completed the modified Questionnaire of Smoking Urges-Brief (QSU-Brief) intended to assess desire or intention to smoke/use an ECIG and anticipation from relief from nicotine/tobacco withdrawal (Wagener et al., 2017). Results indicated that, relative to baseline “second” and “third generation” ECIGs significantly reduced the desire to use an ECIG and anticipation of relief from withdrawal although no significant

between group differences in withdrawal suppression were observed. Limited sample size and lack of experimental control over device and liquid characteristics may be two explanations for the lack of between group differences in subjective experience. Another may be that these particular devices influence other subjective effect effects (e.g., "satisfaction") and non-nicotine sensory stimuli (e.g., "throat hit", sight of smoke-like aerosol) that were not evaluated. Indeed, anecdotal reports of users of "sub-Ohm" devices reveal several subjective effects that have not been explored empirically.

To better understand "sub-Ohm vaping", in November, 2017 a Google search for this term was conducted, yielding over 1 million hits. A non-systematic examination of a portion of these hits revealed a wide range of perceived benefits for the use of these devices including warmer vapor (i.e., aerosol), larger "cloud" production (i.e., more aerosol), greater "throat-hit", and more intense flavor. Specifically, several online ECIG vendors describe the benefits of "sub-Ohm vaping" in blogs and articles (www.halocigs.com/blog/5-benefits-sub-ohm-vaping/; www.migvapor.com/ecig-news/sub-ohm-vaping-guide/). An examination of one such blog revealed four main benefits including: 1) "bigger vapor production", 2) "smoother hit", 3) "warmer clouds and 4) "intense flavor" (www.vaporesso.com/cloud-community-blog/the-benefits-of-sub-ohm-vaping). Some claims regarding "sub-Ohm" vaping include that these devices "heat up the e-liquid to a higher temperature, making the cloud production greater and thicker in comparison to your traditional vape pen" and that these devices provide a "tasting experience far superior to that of devices that use a higher resistance" (www.vaporesso.com/cloud-community-blog/the-benefits-of-sub-ohm-vaping). Many of these claims are corroborated by individual ECIG users via popular ECIG-related discussion forums. According to one user: "I personally vape sub-ohm because I like the big clouds, I prefer the

taste..." (Arnie H; www.e-cigarette-forum.com/forum/threads/sub-ohm-vaping-pros-cons-why.464546/). Despite the prevalence of these anecdotal claims, the subjective effects of using "sub-Ohm" devices have not been examined empirically.

In sum, few studies have investigated how low resistance coils (i.e., <1 Ω) influence user plasma nicotine concentration and subjective effects, two factors that can influence the abuse potential of ECIGs (Benowitz, 2008; Glautier, 2004). Finally, there are no reports to date that have examined user puff topography when using "sub-Ohm" devices.

The Present Study

In this within-subject clinical laboratory study, experienced ECIG users underwent four conditions that differed by ECIG coil resistance (0.5 Ω or 1.5 Ω) and liquid nicotine concentration (3 or 8 mg/ml). In each study session, the ECIG battery (Kanger SUBOX), the ECIG-tank (Subtank mini), voltage (4.5 V), liquid solvent ratio (30PG:70VG) and liquid flavor (e.g., honeydew pear) were held constant. By manipulating ECIG coil resistance, device power varied across conditions: 40.5 W in the 0.5 Ω condition and 13.5 W in the 1.5 Ω condition. In each session, participants underwent a 10-puff directed bout (30 sec IPI) and a 60-minute *ad libitum* bout. The primary outcome measures include nicotine delivery, subjective effects, and puff topography. Secondary measures include heart rate (HR) and amount of liquid consumed.

Statement of the Hypothesis

The four main hypotheses of the present study were as follows: 1) the greatest increases in nicotine delivery would be observed when the 8 mg/ml liquid nicotine concentration was paired with the 0.5 Ω resistance coil, 2) independent of liquid nicotine concentration, greater nicotine delivery would be observed with use of the 0.5 Ω coil relative to the 1.5 Ω coil, 3) the most pronounced abstinence symptom suppression would be observed when the 8 mg/ml liquid

nicotine concentration is paired with the 0.5 Ω resistance coil as this combination would likely deliver the most nicotine and 3) independent of nicotine concentration, use of the ECIG with the 0.5 Ω resistance coil would produce more pleasurable subjective effects (e.g., increases in "throat hit", "taste" and aerosol production) relative to the 1.5 Ω resistance coil.

Method

Participant Selection

Thirty-two ECIG-experienced users, who were volunteers from the local community, completed this within-subject study. An *a priori* power analysis indicated that this number of participants would be sufficient to obtain a power of at least 0.80. This sample size was estimated for three key outcome measures including plasma nicotine, subjective effects and puff duration. Sample size was estimated by first estimating effect size, using means and standard deviations from previous ECIG-related studies, followed by a power analysis for each of the three key outcome measures using G*Power software (Faul, Erdfelder, Buchner, & Land, 2009).

For plasma nicotine, effect sizes were derived using data from two studies described elsewhere in this document (i.e., Hiler et al., 2017 and Wagener et al., 2017) for which effect sizes were large (fs ≥ 0.64; Cohen, 1969). Given the methodological differences across the aforementioned studies and the present study, a power analysis for plasma nicotine was conducted using a more conservative, medium effect size (i.e., f = .25; Cohen, 1969). Thus, for the outcome measure of plasma nicotine, to determine medium effects within groups (assuming correlations across repeated measures of 0.6-0.8 as seen in Hiler et al, 2017), a sample size of 19 participants was required (80% power, alpha < .05).

For subjective measures, data from a study in which device power was varied from 6 to 10 W (Farsalinos et al., 2017) were used to estimate effect sizes for items such as "aerosol" (f =

0.4) and “throat hit” (f = 0.24). Moreover, one previous study reported large effect sizes for some items such as “urge” (f = 0.58), “craving” (f = 0.54) whereas other items, such as “pleasant” (f = 0.22) had small to medium effects (Hiler et al., 2017). Given the range of effect sizes reported in previous studies, a sample size from 10-24 participants was necessary to detect effects on several subjective outcomes (80% power, alpha < .05).

For the outcome of puff duration, effect size derived using data from a previous study (Farsalinos et al., 2017) were large (f = 0.44) as were effect sizes reported from another study (f = 0.39; Hiler et al., 2017). Therefore, to determine medium effects within groups, a sample size of nine participants was required (80% power, alpha < .05). Collectively for all key outcome measures, results revealed that for within-subject analyses upwards of 24 participants would be required to achieve power > 80% given a Type I error rate of 0.05 and assuming correlations across repeated measures of 0.6-0.8. Given that no studies to date have examined the research question proposed in the present study, a sample size of 32 was proposed to ensure enough power to detect a variety of subjective effects, with the understanding the study may be over-powered on other outcome measures (e.g., plasma nicotine, puff topography).

Study participants were recruited by Institutional Review Board (IRB)-approved advertisements and/or word-of-mouth. Experimental sessions took place at the Clinical Behavioral Pharmacology Laboratory (CBPL) located on Virginia Commonwealth University’s (VCU) medical campus. The CBPL is part of VCU’s Center for the Study of Tobacco Products (CSTP). Participants were eligible for the study if they were healthy, 18-55 years of age, and if they were experienced ECIG users meaning: they self-reported use of ≥ 1 ml of ECIG liquid daily, use of an ECIG liquid nicotine concentration ≥ 3 mg/ml, had been using their ECIG for ≥ 3 months (similar to Spindle et al., 2018) and if they provided a semi-quantitative urine cotinine

test result of ≥ 3 at screening (NicAlertTest) providing objective verification of nicotine use. Lastly, to be eligible participants had to report use of < 5 tobacco cigarettes daily and use of other tobacco products (e.g., cigars or waterpipe) no more than 3 times per week (as in Vansickel & Eissenberg, 2013).

Individuals were excluded from participation in the study if they self-reported a history of chronic health problems, psychiatric conditions, regular use of a prescription medication (except vitamins or birth control), weighing ≤ 110 pounds, marijuana use > 10 and alcohol use > 25 days in the past 30, and use of other illicit drugs (e.g. cocaine, opioids, benzodiazepines, and methamphetamine) in the past 30 days. To control for effects of ECIG-liquid flavor novelty across participants, those individuals who listed the specific study flavor (e.g., honeydew pear) as one of their top five preferred flavors were excluded to ensure the flavor was similarly unique for all participants (similar to Buchhalter et al., 2001). Women were excluded if they tested positive for pregnancy (by urinalysis) at screening.

Screening and Informed Consent Procedures

Potential participants underwent a two-part screening process. First, prospective participants completed a brief phone interview or online survey (via the CBPL's secure online screening tool) where they were asked about their health, tobacco use, and ECIG use; questions contained in the telephone screen were identical to those completed online. Individuals meeting the initial study requirements were invited to come to the laboratory for an in-person screening assessment where informed consent was obtained. Following the informed consent process, individuals provided additional information about their health, tobacco use, ECIG use, and illicit drug use to confirm eligibility; demographic questionnaires and nicotine dependence measures were also administered at this time though these questionnaires did not impact eligibility. Next,

participants expired air CO concentration was recorded and participants provided a urine sample for semi-quantitative urine cotinine test (NicAlertTest); women also had urine tested immediately for pregnancy (Accutest Value hCG urine pregnancy test, Jant Pharmaceutical Corp). Lastly, participants who were determined to be eligible were familiarized with study measures and equipment.

Demographic Summary

A total of 38 community volunteers met the initial study screening criteria via a telephone or online interview and provided informed consent for the study. Of these 38 individuals, six did not complete the study and were not included in the final analyses. Of the six individuals who did not complete the study, two were ineligible during the in-person screening (i.e., one for an undisclosed medical condition/prescription medication use and the other for urinary cotinine test indicating no regular nicotine use). The remaining four individuals began the study but were discontinued for failure to attend study sessions (n = 2) and lack of venous access (n = 2).

Thirty-two community volunteers completed all four study sessions and were included in analyses. As indicated by Table 1, eight of the 32 participants who completed the study were women and the participant sample had a mean (SD) age of 25.6 years (7.1). Nineteen participants self-identified as White/Caucasian, seven as Black/African American, four as Asian, and 2 as other. With regard to self-reported ethnicity, 28 participants identified as Not Hispanic or Latino and four identified as Hispanic or Latino. The participants who completed the study had been using ECIGs for a mean (SD) duration of 2.6 years (1.5), with a liquid nicotine concentration of 27.3 mg/ml (25.5), and used 4.8 ml (5.4) of liquid per day. Current cigarette smoking was reported by two participants who reported smoking one cigarette daily. Consistent

with participants' self-reported current cigarette smoking status, mean (SD) expired air CO during screening was 2.2 ppm (1.8). For the Fagerström Test for Nicotine Dependence (scores ranging from 0-10) participants had mean (SD) scores of 4.6 (2.0) indicating low to moderate dependence (Heatherton, Kozlowski, Frecker, & Fagerström, 1991). For the PROMIS-E dependence measure (with scores ranging from 0-4), mean (SD) scores were 1.9 (0.8). Finally, 21 participants reported lifetime experience using a "sub-Ohm" device (coil resistance <1 Ω) and 17 reported current (i.e., past 30 day) use of a "sub-Ohm" device. Information gathered from participants at screening regarding their preferred ECIG device settings and liquid characteristics is reported in Table 2.

Table 1.

Demographic and Screening Data for Study Completers (N = 32)

	Mean or N	SD
Number Female	8	
Number White/Caucasian	19	
Age (years)	25.6	7.1
Screen CO	2.2	1.8
Volume ECIG liquid used/day (ml)	4.8	5.4
Liquid concentration (mg/ml)	27.3	25.5
Duration ECIG use (years)	2.6	1.5
Current use of 'sub-Ohm' device	17	
Ever use of 'sub-Ohm' device	21	
Current cigarettes/day	0.1	0.3
Former smokers	27	
Past number of cigarettes/day[a]	9.1	8.7
Months abstinent from cigarettes[a]	22.9	20.5
PROMIS-E [b]	1.9	0.8
Fagerström TND[c]	4.6	2.0

[a]Data from 25 participants who are former cigarette smokers but are not current smokers.

[b]The PROMIS Nicotine Item Banks for use with ECIGs (Morean et al., 2018).

[c]The Fagerström Test for Nicotine Dependence (Heatherton et al., 1986).

Table 2.

Own Brand ECIG Device and Solution Characteristics (Based on Product Labeling and Manufacturer Information).

Participant	ECIG model	Device Wattage	Coil Resistance	Liquid Flavor	Nicotine Concentration (mg/ml)
1	Smok	80	0.5	Strawberry	3
2	I-Joy	42	0.4	Strawberry	6
3	Kangertech	60	0.6	Strawberry cheesecake	12
4	Kangertech	30	0.7	Watermelon	3
5	Kangertech	unknown	unknown	Sour grape	3
6	Voopoo	unknown	0.2	Mango	3
7	Juul	8.1	1.6	Mango	59
8	Juul	8.1	1.6	Mint	59
9	Fuchai	30	0.2	Strawberry	18
10	Juul	8.1	1.6	Mango	59
11	Juul	8.1	1.6	Grape	59
12	Juul	8.1	1.6	Tobacco	59
13	Kangertech	unknown	unknown	Strawberry Lemonade	6
14	Juul	8.1	1.6	Mint	59
15	Kangertech	20	0.5	Mallowman	60
16	Juul	8.1	1.6	Mint	59
17	Smok	70	0.3	Mint	3
18	Juul	8.1	1.6	Mint	59
19	Juul	8.1	1.6	Mint	59
20	Kangertech	100	0.2	Strawberry & Menthol	30
21	Voopoo	50	0.4	Fruit	3
22	Smok	35	0.4	Custard	6
23	Smok	70	0.2	Pineapple-Strawberry	6
24	Smok	44.4	0.4	Strawberries & cream	12
25	Wismec	85	0.2	Berry	3
26	Suorin	13	1.3	Watermelon Mojito	60
27	Suorin	13	1.3	Raspberry Lemonade	50
28	Smok	unknown	unknown	Mango	30
29	Kangertech	35	0.7	Strawberry	3
30	Juul	8.1	1.6	Mango	59
31	Vaporesso	16	1.2	Fruit	12
32	Smok Baby Stick	58	0.3	Churro	3

Materials

For each of the four study sessions, participants used a "Kangertech SUBOX" ECIG battery, attached to a "Subtank mini" tank with either a 0.5 Ω or 1.5 Ω coil attached; Kangertech Stainless Steel Organic Cotton coils were used and are made of organic cotton wick and Nichrome wire. The "Subtank mini" has an ECIG liquid capacity of 4.5 ml and was pre-filled with 3.5 ml of ECIG liquid in Honeydew Pear flavor comprised of 30% PG: 70% VG; flavor and PG:VG ratio were identical across sessions. Honeydew Pear flavor was selected because fruit is a commonly preferred liquid flavor (e.g., Farsalinos et al., 2013; Kim et al., 2016; Soneji et al., 2019). Depending on the study condition, the nicotine concentration of the ECIG liquid loaded into the "Subtank mini" was either 3 mg/ml or 8 mg/ml free-base nicotine liquid. All ECIG liquids were made by and purchased from a single ECIG vendor (Avail, Richmond, VA) and nicotine concentrations were verified via GC-MS at VCU (as in Hiler et al., 2017; Lopez et al., 2016; Spindle et al., 2015, 2017, 2018). Prior to the start of each study session, coil resistance was verified using a multimeter (Coil Master 521 TAB v2 Ohm Meter) and the internal multimeter contained within the "SUBOX" device. Coils were included if their resistance was ± 0.1 Ω of the intended resistance for 0.5 Ω and 1.5 Ω coils. Finally, prior to the start of each study session, the organic cotton wick contained within each coil was saturated with ECIG liquid, or 'primed,' as recommended by the product manufacturer (i.e., Kangertech) and online ECIG vendors (Vappora, 2018). Coil 'priming' is recommended to avoid activation of a heating coil that contains dry and unsaturated cotton wick as doing so reportedly results in an unpleasant taste (Vappora, 2018). To 'prime' the coil, a pipet containing ECIG liquid was used to place liquid onto each of the external holes on the side and top of the coil where the cotton wick was exposed; within seconds, the cotton wick absorbed the ECIG liquid. Next, the "Subtank mini"

was assembled, with the coil attached, and filled with 3.5 ml of ECIG liquid allowing the coil to remain submerged in ECIG liquid prior to use. At minimum, each coil was submerged in ECIG liquid for 30 minutes prior to the first ECIG use bout.

Procedures

The study procedure for each of four experimental sessions is illustrated in Figure 3. After the successful completion of screening and informed consent procedures, participants attended the CBPL, located on VCU's medical campus, on four separate days (separated by a minimum of 48 hours to control for carryover effects) for four, single-blind independent laboratory sessions that lasted approximately 3.5 hours each. Sessions were ordered by Latin square and differed only by ECIG coil resistance (0.5 Ω or 1.5 Ω) and liquid nicotine concentration (3 or 8 mg/ml) combination. By manipulating ECIG coil resistance, device power varied across conditions: 40.5 W in the 0.5 Ω condition and 13.5 W in the 1.5 Ω condition. Participants were unaware of study conditions; to ensure participants were blinded to the device settings that are displayed on the battery screen (i.e., resistance, voltage, and wattage) study staff attached the appropriate ECIG coil (0.5 Ω or 1.5 Ω), covered the ECIG's battery screen with opaque tape and placed a rubber cover over the entire device battery prior to each session. The ECIG "tank" was weighed before and after the 10-puff bout as well as before and after the *ad libitum* bout to measure the amount of liquid consumed by each participant using a Mettler Toledo advanced precision balance (Model XP10002S; 0.01g)

At the start of each study session, ~12-hour nicotine/tobacco abstinent participants provided expired breath CO concentration in order to verify abstinence from combustible tobacco (≤ 7 ppm, within the range recommended by Cropsey, Elridge, Weaver, Villalobos, & Stitzer, 2006; Devici, Devici, Azik, and Ozan, 2004; Jarvis, Tunstall-Pedoe, Feyerabend, Vesey,

& Saloojee, 1987). Under normal conditions, ECIGs do not produce CO and therefore abstinence from non-combustible tobacco products was verified retrospectively using a baseline plasma nicotine concentration cut-off of ≤ 5 ng/ml (as in Hiler et al., 2017; Maloney et al., 2019; Spindle et al., 2018). Because non-compliance with pre-session ECIG/nicotine abstinence has been documented in previous studies that include experienced ECIG users (Hiler et al., 2017; Spindle et al., 2018) the present study also included a one-hour waiting period prior to the start of the study session to ensure at least 60 minutes of verified nicotine/tobacco abstinence (as in Spindle, 2018) and a bogus pipeline saliva test in an effort to improve participant compliance with abstinence requirements (as in Donny & Jones, 2009; Rose & Behm, 1991). Given the short half-life of nicotine (i.e., 1-2 hours; Benowitz et al., 2008), the one hour waiting period was intended to reduce baseline plasma nicotine concentration of participants' who were not 12 hours abstinent while increasing their chances of experiencing nicotine/tobacco abstinence symptoms prior to the onset of the study session. Since instituting the one hour waiting period, abstinence requirements in recent clinical laboratory evaluations of ECIGs have improved, with one study reporting ~90% of participants being abstinent prior to all study sessions (i.e., participants baseline plasma nicotine concentrations ≤ 5 ng/ml; Spindle, 2018).

Immediately following the completion of the measurement of expired air CO, collection of saliva, and completion of the one-hour waiting period, the monitoring of physiological responses (HR and BP) began. Next, a nurse inserted an intravenous catheter into the participants forearm vein. Thirty minutes after catheter insertion, 7 ml of blood was sampled (-5 min) and participants completed computerized baseline subjective questionnaires assessing nicotine/tobacco abstinence symptoms (see Figure 3). After completing baseline subjective questionnaires, participants completed a directed ECIG use bout (0 min) consisting of 10 puffs

over the course of 5 minutes (30 sec IPI). Immediately following the tenth puff, another 7 ml of blood was sampled (5 min) and participants responded to subjective questionnaires again. After 60 additional minutes, 7 ml of blood was sampled and participants responded to subjective questionnaires (65 min). Five minutes after the third blood sample was collected (70 min), participants began a 60-minute *ad libitum* bout in which they were instructed to puff on the ECIG as much as they liked and whenever they liked. During the *ad libitum* bout, two additional 7 ml blood samples were taken, one every 20 minutes, followed by subjective questionnaires (90 and 110 min). Immediately after the *ad libitum* bout is completed, the sixth and final blood sample occurred followed by administration of subjective questionnaires (130 min). Five minutes after the final blood sampling the catheter was removed, and participants were compensated for their time abstaining prior to the study session and for their time participating in the study (US $75 after first session, $75 after second, $150 after the third and $200 after the fourth).

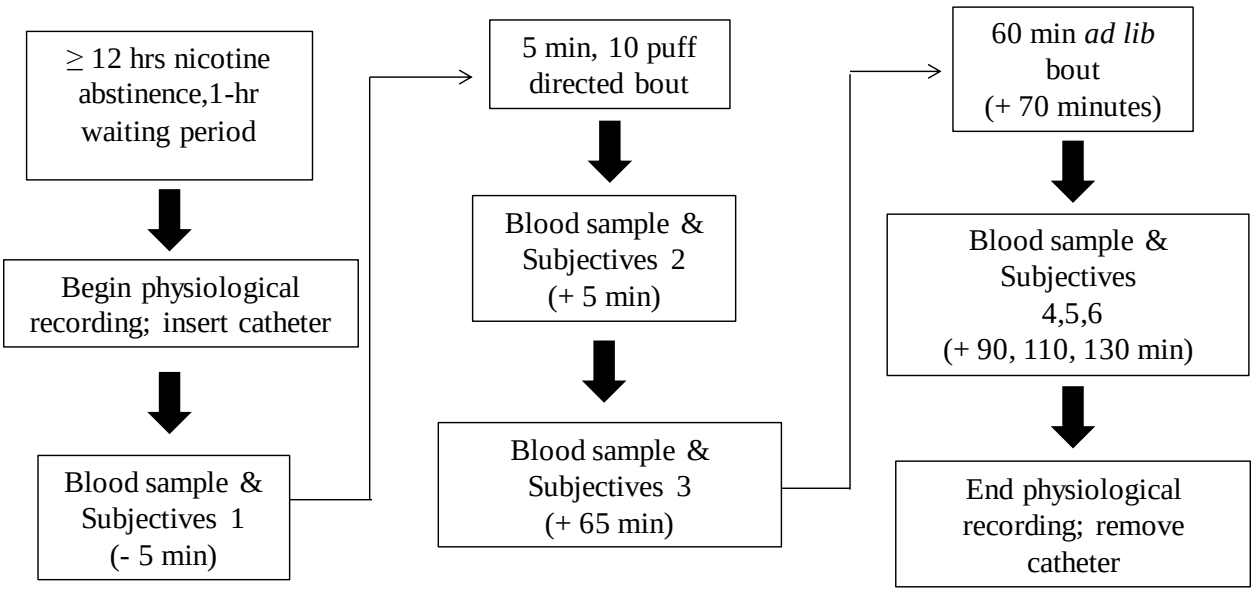

Figure 3. The session procedure involved participants visiting the laboratory for four, 3.5-hour sessions. Participants were asked to arrive for study sessions having abstained from nicotine/tobacco for ≥12 hours. Abstinence from combustible tobacco products was verified by pre-session CO recording of ≤ 7 ppm. After participants met this requirement and completed a 1-hour waiting period, physiological monitoring commenced, an intravenous catheter was inserted into a forearm vein, and the session began.

Participant Safety and Rights

The methods and procedures involved in the present study have been conducted at the CBPL for over 20 years involving users of various tobacco products (e.g., Buchhalter et al., 2001; Breland et al., 2002; Blank et al., 2009; Cobb et al., 2010; Blank et al., 2011; Lopez et al., 2016; Maloney et al., 2019), including ECIGs (e.g., Vansickel et al., 2010; Hiler et a., 2017; Spindle et al., 2015, 2017, 2018). The procedures and methods herein pose no more than minimal risk to participants, meaning that the likelihood and extent to which participants may experience discomfort or harm is no greater than that which they may encounter in daily life or during routine physical or psychological tests (WPP #: VIII-2; http://www.research.vcu.edu/human_research/irb_wpp/VIII-2.htm). Interested individuals were notified of the potential risks in participating in the study during the informed consent process. As described in the consent form, the risks associated with ECIG/nicotine use (e.g., sweating, lightheadedness, dizziness, nausea, and nervousness) are encountered in daily life of these experienced ECIG users. Pre-session ~12-hour nicotine/tobacco abstinence may result in discomfort but is not medically dangerous and does not pose a threat to safety. Also, the blood sampling procedure, which occurs via an intravenous catheter, involves minimal risk of bruising and/or infection. The risks associated with blood sampling procedures are minimized by the laboratory's trained nurse and the use of aseptic nursing procedures and sterile, disposable equipment.

Trained staff, including a registered nurse and on-call medical monitor, ensured protection of participants' safety and rights throughout the study. Physiological monitoring of HR and blood pressure (BP) occurred in real-time by an experienced registered nurse and trained personnel. Research personnel were trained to notify the research nurse if HR elevated above

120, if systolic BP exceeded 150, or if diastolic BP exceeded 100 so that the participant could be monitored more closely. Sessions were stopped if a participant's HR elevated above 120 or dropped below 50 for more than 5 minutes, if systolic BP elevated above 170 more than twice, or if diastolic BP elevated above 100 more than twice after repositioning the BP cuff. Data were maintained with professional standards regarding confidentiality. Data were identified using a unique alphanumeric code only and stored in locked cabinets and/or within locked laboratory rooms only available to CBPL staff.

Outcome Measures

Physiological Measures. All blood samples were centrifuged, stored at -70°C, and subsequently sent to VCU's Bioanalytical Analysis Core Laboratories to be analyzed for nicotine concentration (limit of quantitation or LOQ = 2 ng/ml; see Breland et al., 2006). Using Criticare Systems model 507, fitted with pulse oximeter, HR and BP were monitored every 20 sec. Participants' expired air CO concentration were measured via a BreathCO monitor (Vitalograph, Lenexa, KS).

Subjective Questionnaires. Six subjective questionnaires were administered and five of those questionnaires (Hughes-Hatsukami, Direct Effects of Nicotine Scale, Direct Effects of ECIG Use, gLMS and the LHS) were administered using a computerized visual analog scale (VAS) that consisted of a word or phrase centered on a horizontal line with "not at all" on the left and "extremely" on the right. Participants recorded their responses by clicking a mouse cursor onto any point on the horizontal line, and scores were expressed as a percentage of total line length (0-100). Items on the Hughes-Hatsukami were modified from the original versions intended for tobacco cigarette smokers, such that in the present study, the words "cigarette" or "smoking" were replaced by "e-cigarette" or "vaping." Three questionnaires (Hughes-Hatsukami

withdrawal scale, Questionnaire of Vaping Craving, Direct Effects of Nicotine) were administered at six separate time points during each experimental session. The Direct Effects of ECIG Use scale were administered at five separate time points, omitting baseline as participants had not yet sampled the product at that time. The gLMS and LHS were administered at the two time points immediately after the end of each ECIG bout.

Hughes-Hatsukami Withdrawal Scale. This 11-item VAS measure was intended to assess nicotine abstinence symptom suppression and included the following items: "Anxious," "Craving an e-cigarette/nicotine," "Depression," "Difficulty concentrating," "Drowsy," "Hunger," "Impatient," "Irritable," "Restlessness," "Desire for sweets," and "Urges to use an e-cigarette" (Hughes & Hatsukami, 1986). The present study omits two items from the original: "Increased eating" and "Insomnia/Disturbed sleep" (as in Breland et al., 2002; Buchhalter et al., 2005; Hiler et al., 2017; Spindle et al., 2018; see appendix A).

Questionnaire of Vaping Craving. This 10-item measure assessed abstinence symptom suppression using the following items: "I have a strong desire for an e-cigarette right now," "I have an urge for an e-cigarette," "All I want right now is an e-cigarette," " I am missing vaping right now," "I am craving an e-cigarette right now," " I need to vape now," " I am going to vape as soon as possible," "Nothing would be better than vaping right now," "Vaping would make me happier right now" (Cox, Tiffany, & Christen, 2001; Dowd, Courtney, Motschman, & Tiffany, 2018; see appendix B). Participants were asked to rate each phrase on a 7-point scale ranging from 0 (Strongly disagree) to 7 (Strongly agree). The items from this scale formed two factors: Factor 1 (intention to use an e-cigarette) and Factor 2 (anticipation of relief from abstinence symptoms).

Direct Effects of Nicotine (Modified). This 9-item measure evaluated nicotine-related

effects associated with nicotine delivery from an ECIG and included the following items: "Confused," "Headache," "Heart Pound," "Lightheaded," "Nauseous," "Nervous," "Salivation," "Sweaty," and "Weak" (Evans et al., 2006). The present study omits one item from the original: "Dizzy" as this item is redundant with other questionnaires that were administered in the present study (see appendix C).

Adapted Direct Effects of ECIG Use. This 14-item VAS measure, adapted from the "Direct Effects of Tobacco" scale, was developed using items reported in studies assessing the subjective effects of smoking (e.g., Foulds et al., 1992; Pickworth, Bunker, & Henningfield, 1994) and has been modified for the present study to include four additional questions. This scale assessed the following subjective effects of ECIG use: "Did the e-cigarette make you feel more awake?," " Did the e-cigarette help calm you down?," "Did the e-cigarette help with concentration?," "Did the e-cigarette make you dizzy?," "Was the e-cigarette pleasant?," "Did the e-cigarette reduce hunger?," "Would you like another e-cigarette right now?," "Was the e-cigarette satisfying?," "Did the e-cigarette make you sick?," and "Did the e-cigarette taste good?" In addition to the original 10 items, the present study also included the four following questions: "Did you enjoy the smell of the e-cigarette?", "How similar were the puffs you took to your own e-cigarette?", "Did the e-cigarette produce visible vapor or cloud?" (as in Farsalinos et al., 2017), "How important was the presence of the vapor or cloud to you?" (see appendix D).

General Labeled Magnitude Scale. This category-ratio scale contains seven semantic labels that increase in sensitivity including: "no sensation", "barely detectable", "weak", "moderate", "strong", "very strong", and "strongest imaginable sensation of any kind." As in Rosbrook and Green (2016), participants were asked to rate the overall sensation of the flavoring, the harshness/irritancy, and the throat hit immediately after each ECIG bout. In the

present study, participants also were asked to rate the overall sensation of the warmth of the aerosol or "vapor" immediately after each bout. Responses were coded on a 0-100 scale (see appendix E).

Labeled Hedonic Scale. This category-ratio scale contains ten semantic labels, intended to assess participant liking or disliking of sensations experienced in the gLMS. Semantic labels include: "most liked sensation imaginable", "like extremely", "like very much", "like moderately", "like slightly", "dislike slightly", "dislike moderately", "dislike very much", "dislike extremely" and "most disliked sensation imaginable" that assess how much participants like or dislike the four items assessed on the gLMS (Lim et al., 2009; see appendix F).

Puff Topography. Using an ECIG topography instrument developed and manufactured at the American University of Beirut (AUB; see Spindle et al., 2015), puff topography was measured throughout the 10-puff directed bout and the 60-minute *ad libitum* bout. Puff topography variables included: puff duration, volume, flow rate, number and IPI. Prior to each study session, the mouthpiece was calibrated using a customized automatic digital flow calibrator.

Amount of Liquid Consumed. To measure the amount of liquid consumed during each ECIG use bout (i.e., the 10-puff directed and 60-minute *ad* libitum bout), each "tank" was weighed at the beginning and end of each bout. The difference between the pre and post ECIG use "tank" weight represented the total amount of liquid consumed (in grams) for each bout (as in Wagener et al., 2017). In order to derive the amount of liquid consumed, per bout in milliliters, the total amount of liquid consumed in grams from each bout was divided by the density of the liquid being used. Because each session includes one 10-puff directed bout and one *ad libitum bout*, two liquid consumption variables were generated for each session.

Data Analysis Plan

Data preparation. For plasma nicotine, values below the LOQ were replaced with the LOQ (2 ng/ml), as this is a more conservative approach compared to identifying each value below the LOQ as zero (as in Hiler et al., 2017; Lopez et al., 2016; Maloney et al., 2019; Spindle et al., 2015, 2017, 2018; Vansickel et al., 2010). In addition, participants with a baseline plasma nicotine concentration higher than 5 ng/ml were excluded from analyses, as values > 5 ng/ml imply they have failed to abstain from nicotine/tobacco. Prior to analysis, HR data were averaged for the five minutes during the directed bout and prior to each blood sampling (7 values total). The puff topography software used flow rate (i.e., puff velocity) data to produce the following topography measures: puff duration, puff volume, mean flow rate, puff number, and IPI (see Shihadeh, Azar, Antonios, & Haddad, 2004). Prior to data analysis, the software conducted a data cleaning procedure to correct for transducer noise. The data cleaning procedure combined two or more puffs separated by less than 100 ms into a single puff and delete any puffs less than 300 ms. Remaining data for each topography variable was averaged for each participant.

Data Analysis. Statistical analyses for all outcome measures were conducted using IBM SPSS (Version 24.0). Repeated measures analysis of variance (ANOVAs) were used to examine plasma nicotine, HR, puff topography, subjective effects, and total amount of liquid consumed. Four (condition) by six (time) repeated measures ANOVA were used to examine plasma nicotine and HR data. The Hughes-Hatsukami, Questionnaire of Vaping Craving,and Direct Effects of Nicotine scales were examined using four (condition) by six (time) repeated measures ANOVA. The Direct Effects of ECIG-use scale were examined using a four (condition) by five (time) repeated measures ANOVA as the baseline time point was omitted from analysis because participants had not sampled the product at baseline. The gLMS and LHS were examined using

four (condition) by two (time) repeated measures ANOVA. Separate ANOVAs were conducted to examine each subjective questionnaire item (or factor in the case of the Questionnaire of Vaping Craving) individually. For puff topography, data were averaged across the 60-minute *ad libitum* bout to produce a single value for each variable. Each puff topography variable was analyzed using a four (condition) by two (time) repeated measures ANOVA.

ANOVAs are susceptible to violations of assumptions of sphericity. Sphericity violations occur when the variances between all combinations of related groups are unequal. Violations to sphericity can result in an increase in the Type I error rate if not corrected. The Huynh-Feldt (1976) procedure is a correction generated to adjust for sphericity violations. For all repeated measures factors, significance levels were adjusted for potential violations of sphericity using Huynh-Feldt corrections (Huynh & Feldt, 1976). For all outcome measures, within-subject comparisons were made using Tukey's Honestly Significant Difference (HSD) test, based on the studentized range distribution, to compare all possible pairs of means (Tukey, 1949).

Prior to conducting the main study analyses for plasma nicotine, HR, puff topography, subjective effects, and amount of liquid consumed as described above, plasma nicotine data were first inspected to ensure participants had complied with the study requirement of $\geq$ 12-hours abstinence from all nicotine/tobacco containing products prior to each session. Participants whose baseline plasma nicotine concentrations were 5.0 ng/ml or higher were considered to be not abstinent (as in Hiler et al., 2017; Spindle et al., 2018; Maloney et al., 2019). Of the 32 participants who completed the study, three were considered to have not abstained prior to at least one experimental session (one participant did not abstain prior to one of their four sessions, one did not abstain prior to two study sessions and the remaining one participant did not abstain prior to three of four sessions). To determine whether non-compliance with abstinence

requirements by these three individuals influenced study outcomes, analyses on all outcome measures were conducted with and without these three individuals and the two sets of results were compared. Overall, exclusion of these individuals did not influence study results. Of the 50+ repeated measures ANOVAs conducted with and without non-abstinent participants, results only differed for two subjective items when these individuals were excluded. Given these results, the three non-abstainers were included in analysis as their exclusion did not influence the majority of results and their inclusion in final analysis would increase sample size and improve statistical power.

Results

This within-subject, clinical laboratory study examined the individual and combined influence of ECIG coil resistance and liquid nicotine concentration on various ECIG-related outcomes. Results from all outcome measures including plasma nicotine, HR, subjective measures, puff topography, and amount of liquid consumed are described below. Results from statistical analyses (main effects and interactions) for all physiological and subjective measures are summarized in Table 3.

Physiological Measures

Plasma Nicotine. Figure 4 depicts the mean plasma nicotine results for each condition and study timepoint. As indicated in Table 3, a significant condition by time interaction [F (15, 465) = 3.2, $p < .01$] was observed. Also, significant main effects of condition [F (3, 93) = 11.5, $p < .001$] and time [F (5, 155) = 41.5, $p < .001$] were observed for plasma nicotine.

Post-hoc analyses (Tukey's HSD) revealed that mean (SD) plasma nicotine concentrations increased for the 8 mg/ml+0.5Ω combination from 2.7 ng/ml (2.6) at baseline to 10.2 ng/ml (8.2) following the 10-puff bout and to 15.4 ng/ml (11.7) at the conclusion of the *ad libitum* bout (Tukey's HSD, ps < .05). For the 3 mg/ml+0.5Ω combination, mean (SD) plasma nicotine concentrations increased from 2.5 ng/ml (1.5) at baseline to 7.0 ng/ml (5.0) following the 10-puff bout and to 13.2 ng/ml (11.0) at the conclusion of the *ad libitum* bout (i.e., 60 minutes; Tukey's HSD, ps < .05). For the 8 mg/ml+1.5Ω combination, mean (SD) plasma nicotine concentration increased from 2.5 ng/ml (1.9) at baseline to 7.1 ng/ml (8.7) following the 10-puff bout and to 11.4 ng/ml (8.5) at the conclusion of the *ad libitum* bout (Tukey's HSD, ps < .05). For the 3mg/ml+1.5Ω combination, there were no significant increases in plasma nicotine concentration immediately following the 10-puff bout, though plasma nicotine concentrations increased significantly from 2.5 ng/ml (1.5) at baseline to 7.7 ng/ml (6.0) at the conclusion of the

ad libitum bout (Tukey's HSD, *p*s < .05). Overall, plasma nicotine concentrations were greater at the conclusion of the *ad libitum* bout relative to the 10-puff ECIG use bout for each device and liquid combination.

Additional post-hoc testing was conducted to examine the influence of condition on plasma nicotine concentration for the timepoints immediately following the 10-puff directed bout and at 20, 40 and 60-minutes of the *ad libitum* bout. These analyses revealed significant differences in plasma nicotine concentration across conditions for the 10-puff directed bout and at the conclusion of the *ad libitum* bout. Following the 10-puff directed bout, mean (SD) plasma nicotine concentrations of 10.2 ng/ml (8.2) for the 8 mg/ml+0.5Ω combination were significantly greater than mean plasma nicotine concentrations of 7.0 ng/ml (5.0) for the 3 mg/ml+0.5Ω combination, 7.1 ng/ml (8.7) for the 8 mg/ml+1.5Ω combination and 4.6 ng/ml (3.3) for the 3 mg/ml+1.5Ω combination (Tukey's HSD, *p*s < .05). At the conclusion of the *ad libitum* bout, mean (SD) plasma nicotine concentration of 15.4 ng/ml (11.7) for the 8 mg/ml+0.5Ω continued to be significantly higher relative to the mean plasma nicotine concentrations of 11.4 ng/ml (8.5) observed for the 8 mg/ml+1.5Ω combination and 7.7 ng/ml (6.0) observed for the 3mg/ml+1.5Ω combination; no significant differences were observed between the 8 mg/ml+0.5Ω and 3 mg/ml+0.5Ω combination. Altogether, the significant differences in plasma nicotine concentration between the 8 mg/ml+0.5Ω and 3mg/ml+0.5Ω combinations following the 10-puff bout no longer persisted at the conclusion of *ad libitum* puffing, likely explaining the interaction between condition and time.

Table 3.

Statistical Analyses Results for Physiological and Subjective Measures.

Outcome measures	Condition (*C*) *F*	*p*	η^2_p	Time (*T*) *F*	*p*	η^2_p	*C* × *T* *F*	*p*	η^2_p
Plasma Nicotine[a]	11.5	<.001*	0.27	41.5	<.001*	0.57	3.23	<.01*	0.09
Heart Rate[a]	2.53	*ns*	0.06	55.0	<.001*	0.64	1.83	<.05*	0.06
Subjective Measures									
Hughes-Hatsukami[a]									
Anxious	0.68	*ns*	0.02	28.3	<.001*	0.48	0.80	*ns*	0.03
Craving	3.17	<.01*	0.1	26.7	<.001*	0.46	1.10	*ns*	0.03
Depression	1.72	*ns*	0.05	6.30	<.01*	0.17	0.80	*ns*	0.03
Difficult Concentrating	0.71	*ns*	0.02	11.1	<.001*	0.26	0.87	*ns*	0.03
Drowsy	1.71	*ns*	0.05	10.7	<.001*	0.26	1.10	*ns*	0.04
Hunger	2.43	*ns*	0.07	7.29	<.001*	0.19	1.45	*ns*	0.05
Impatient	0.48	*ns*	0.02	11.3	<.001*	0.27	0.66	*ns*	0.02
Irritable	0.29	*ns*	0.01	20.4	<.001*	0.40	1.35	*ns*	0.04
Restless	1.50	*ns*	0.05	6.75	<.01*	0.18	1.10	*ns*	0.03
Sweets	0.83	*ns*	0.03	6.2	<.01*	1.70	0.86	*ns*	0.03
Urge	3.30	<.05*	0.10	31.2	<.001*	0.50	1.40	*ns*	0.04
Vaping Craving[a]									
Factor 1 (Intention)	3.80	<.05*	0.11	34.9	<.001*	0.53	1.55	*ns*	0.05
Factor 2 (Anticipation)	4.18	<.01*	0.12	34.4	<.001*	0.53	1.87	*ns*	0.06
Direct Effects of Nicotine[a]									
Confused	0.32	*ns*	0.01	1.78	*ns*	0.05	0.52	*ns*	0.02
Headache	1.03	*ns*	0.03	0.18	*ns*	0.01	0.91	*ns*	0.03
Heart Pound	2.20	*ns*	0.07	1.38	*ns*	0.04	1.00	*ns*	0.03
Lightheaded	2.30	*ns*	0.07	7.78	<.001*	0.20	2.03	*ns*	0.06
Nauseous	0.47	*ns*	0.02	1.76	*ns*	0.05	0.62	*ns*	0.02
Nervous	0.57	*ns*	0.02	8.23	<.01*	0.02	0.54	*ns*	0.02
Salivation	0.40	*ns*	0.01	1.71	*ns*	0.05	0.50	*ns*	0.02
Sweaty	0.62	*ns*	0.02	1.60	*ns*	0.05	1.30	*ns*	0.04
Weak	0.84	*ns*	0.03	0.51	*ns*	0.02	1.10	*ns*	0.03
Direct Effects of Vaping[b]									
Awake	1.60	*ns*	0.05	3.13	<.05*	0.10	0.60	*ns*	0.02
Calm	1.76	*ns*	0.05	9.58	<.001*	0.24	0.30	*ns*	0.01
Concentrate	1.40	*ns*	0.04	2.79	<.05*	0.08	0.41	*ns*	0.01
Dizzy	2.40	*ns*	0.07	20.1	<.001*	0.40	2.51	<.01*	0.08
Pleasant	4.68	<.01*	0.13	0.48	*ns*	0.02	1.18	*ns*	0.04
Reduce Hunger	1.00	*ns*	0.03	0.55	*ns*	0.02	0.78	*ns*	0.02
Right Now	5.20	<.01*	0.14	14.4	<.001*	0.32	1.00	*ns*	0.03
Satisfying	3.65	<.05*	0.12	0.35	*ns*	0.01	0.78	*ns*	0.03
Sick	0.54	*ns*	0.02	2.40	*ns*	0.07	1.33	*ns*	0.04
Taste Good	3.92	<.05*	0.11	0.57	*ns*	0.02	1.51	*ns*	0.05
Smell of e-cigarette	2.86	<.05*	0.08	0.83	*ns*	0.03	0.63	*ns*	0.02
Puffs Similar	2.66	*ns*	0.08	3.27	<.05*	0.10	0.73	*ns*	0.02
Visible Vapor	7.02	<.01*	0.19	2.69	*ns*	0.08	2.03	*ns*	0.06
Importance Vapor	1.51	*ns*	0.05	0.31	*ns*	0.01	1.21	*ns*	0.04
Gen. Labeled Magnitude[c]									
Flavor	7.57	<.001*	0.20	2.13	*ns*	0.07	0.51	*ns*	0.02
Harshness/Irritancy	13.9	<.001*	0.31	0.14	*ns*	0.01	2.82	<.05*	0.08
Throat Hit	23.7	<.001*	0.43	1.40	*ns*	0.04	3.00	<.05*	0.09
Warmth	13.5	<.001*	0.30	1.20	*ns*	0.03	1.44	*ns*	0.05
Labeled Hedonic Scale[c]									
Flavor	2.52	*ns*	0.08	0.09	*ns*	0.01	0.76	*ns*	0.02
Harshness/Irritancy	2.80	*ns*	0.08	1.40	*ns*	0.04	3.10	<.05*	0.09
Throat Hit	3.00	<.05*	0.09	0.01	*ns*	0.00	3.20	<.05*	0.09
Warmth	2.79	*ns*	0.08	0.38	*ns*	0.01	2.02	*ns*	0.06

Note: ns = not significant.

[a]df C = (3,93); df T = (5,155); df C x T (15, 465).

[b]df C = (3,93); df T = (4,124); df C x T (12, 372).

[c]df C = (3,90); df T = (1,31); df C x T (3, 90).

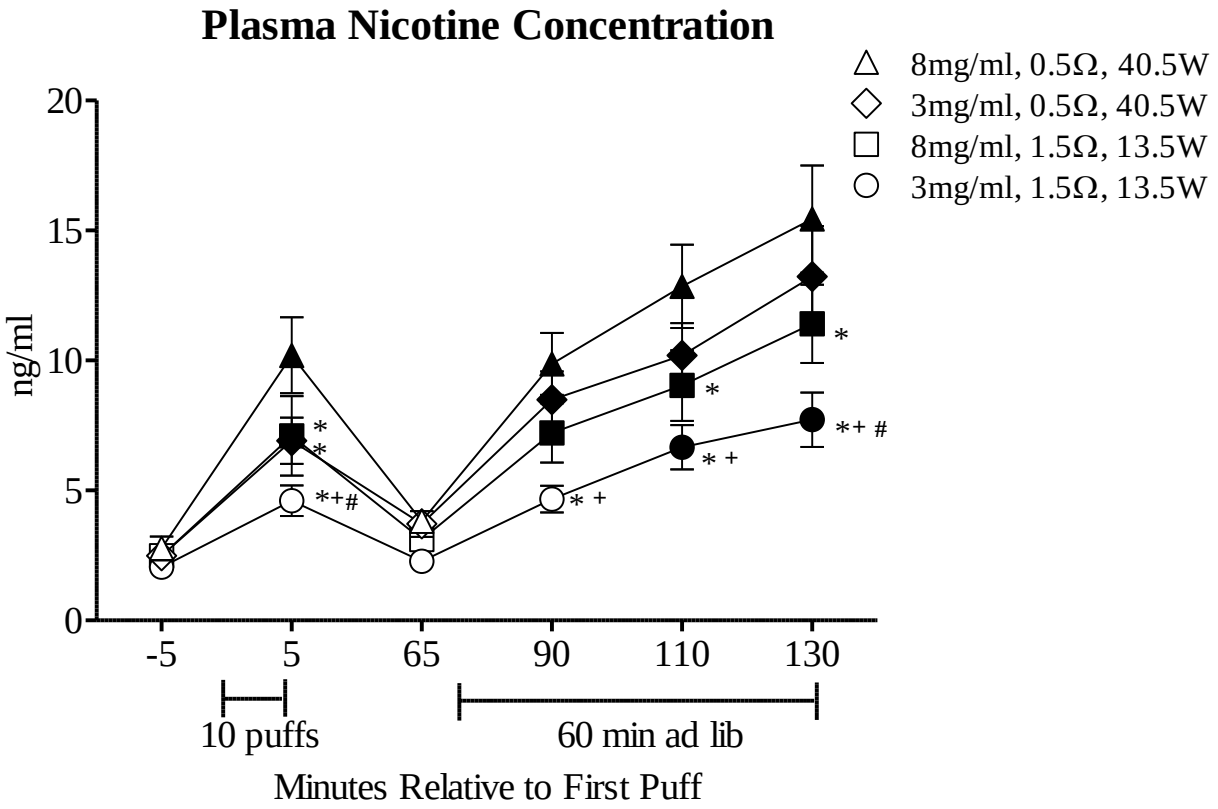

Figure 4. Mean (± SEM) plasma nicotine concentration for experienced ECIG users (N = 32) across conditions. Brackets under the x-axis indicate the 10-puff ECIG use bout and 60-minute *ad libitum* bout. Filled symbols indicate a significant difference from baseline (-5 timepoint), asterisks (*) denote significant differences from 8 mg/ml+0.5Ω combination at that timepoint, crosses (+) indicate significant differences between the 3 mg/ml+0.5Ω and 3 mg/ml+1.5Ω combination at that timepoint. Pound symbols denote significant differences between the 3 mg/ml+1.5Ω and 8 mg/ml+1.5Ω combinations at that timepoint. All *p*'s < .05; Tukey's HSD.

Heart Rate. A significant condition by time interaction [F (15, 465) = 1.8, $p < .05$] was observed for HR as was a significant main effect of time [F (5, 155) = 55.0, $p < .001$].

As displayed in Figure 5, mean (SD) HR increased for the 8 mg/ml+0.5Ω combination from 66.7 bpm (6.4) at baseline to 75.5 bpm (9.3) following the 10-puff bout and to 77.0 bpm (9.1) at the conclusion of the *ad libitum* bout (Tukey's HSD, *p*s < .05). For the 3 mg/ml+0.5Ω combination, mean (SD) HR increased from 68.8 bpm (9.1) at baseline to 75.8 bpm (10.2) following the 10-puff bout and to 78.3 bpm (9.6) at the conclusion of the *ad libitum* bout (Tukey's HSD, *p*s < .05). When using the 8 mg/ml+1.5Ω combination, mean (SD) HR increased from 68.0 bpm (6.3) at baseline to 72.6 bpm (8.2) following the 10-puff bout and to 76.7 bpm (8.5) at the conclusion of the *ad libitum* bout (Tukey's HSD, *p*s < .05). Finally, for the 3 mg/ml+1.5Ω combination, mean (SD) HR increased from 67.3 bpm (7.0) at baseline to 72.3 bpm (6.6) following the 10-puff bout and to 74.0 bpm (8.0) at the conclusion of the *ad libitum* bout (Tukey's HSD, *p*s < .05).

To explain the interaction of condition and time, post-hoc analyses are described for HR across condition for the timepoints immediately following the 10-puff bout and the conclusion of the *ad libitum* bout. Following the 10-puff bout, mean (SD) HR of 75.5 bpm (9.3) was significantly higher for the 8 mg/ml+0.5Ω combination relative to mean HR of 72.6 bpm (8.2) for the 8 mg/ml+1.5Ω combination and mean HR of 72.3 bpm (6.6) for the 3mg/ml+1.5Ω combination; no significant differences were observed for HR between the 8 mg/ml+0.5Ω and 3mg/ml+0.5 Ω combinations (Tukey's HSD, *p*s < .05). Also, after the 10-puff bout, mean (SD) HR was significantly higher for the 3 mg/ml+0.5Ω combination relative to the 3 mg/ml+1.5 Ω combination (Tukey's HSD, *p*s < .05). At the conclusion of the *ad libitum* bout, mean (SD) HR of 77.0 bpm (9.1) for the 8 mg/ml+0.5Ω combination and 78.3 bpm (9.6) for the 3 mg/ml+0.5Ω

combination remained significantly higher relative to mean (SD) HR of 74.0 bpm (8.0) for the 3mg/ml+1.5Ω combination (Tukey's HSD, *p*s < .05). At the conclusion of the *ad libitum* bout there were no significant differences in HR across the 8 mg/ml+0.5Ω and 8 mg/ml+1.5Ω combinations.

Subjective Measures

Hughes-Hatsukami Withdrawal Scale. As indicated in Table 3, no significant interactions were observed for any subjective items in this questionnaire. Significant main effects of time were observed for all 11 subjective items [*F*s (5, 155) ≥ 6.2, *p*s <.01]. Significant main effects of condition were observed for the items "Craving an e-cigarette/nicotine" and "Urges to use an e-cigarette" [*F*s (3, 93) ≥ 3.2, *p*s <.05]. Figure 6 displays the results for "Craving" and "Urge," the two items with the largest *F* values.

For the main effects of time, post-hoc analyses revealed that VAS scores for the items "Anxious," "Craving an e-cigarette/nicotine," "Depression," "Difficultly Concentrating," "Drowsy," "Impatient," "Irritable," "Sweets" and "Urges to use an e-cigarette" were reduced significantly from baseline following the 10-puff bout and at the conclusion of the *ad libitum* bout (Tukey's HSD, *p*s < .05). For example, collapsed across condition, mean (SD) VAS score for the item "Anxious" decreased from 28.0 (26.2) at baseline to 12.9 (15.4) following the 10-puff bout and to 10.1 (14.2) at the conclusion of the *ad libitum* bout (Tukey's HSD, *p*s < .05). Post-hoc analyses revealed no significant reductions in VAS scores from baseline for the subjective items "Hunger" and "Restlessness".

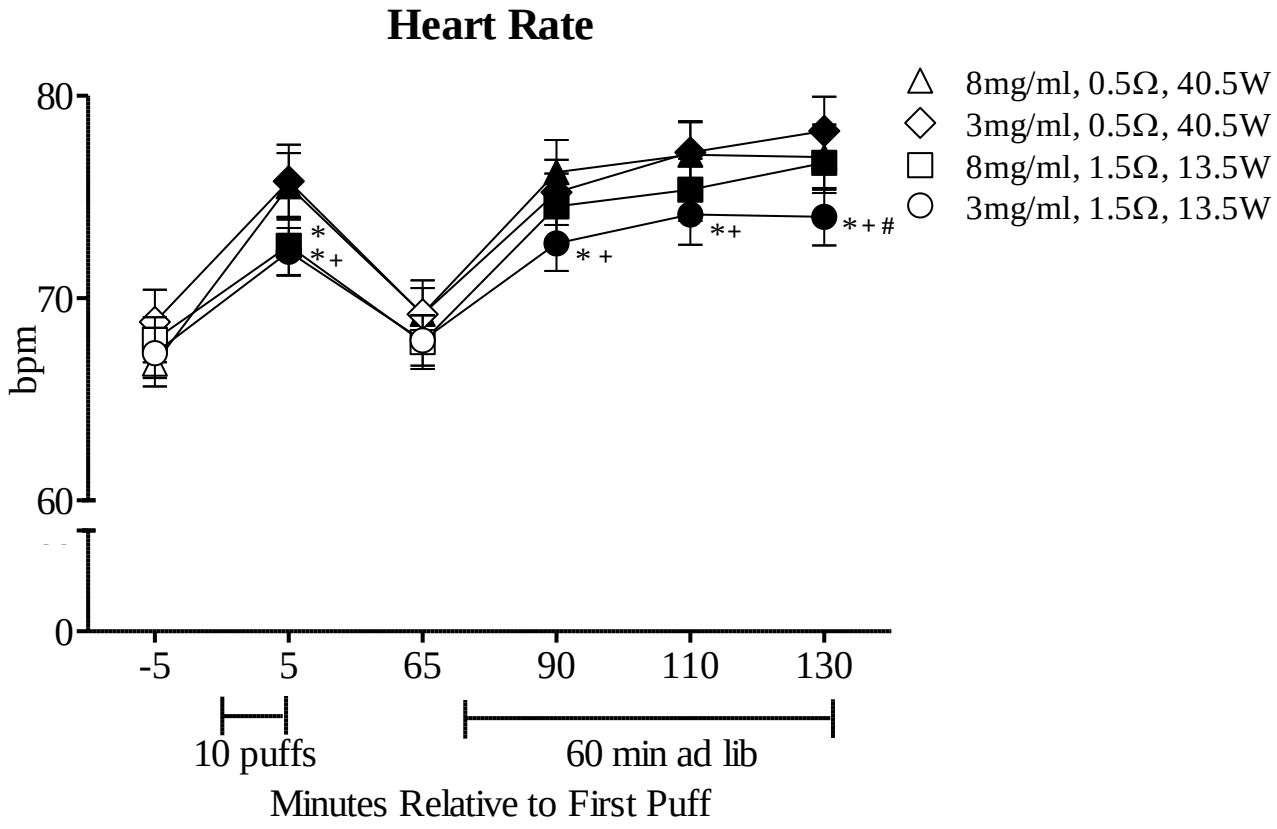

Figure 5. Mean (± SEM) HR (bpm) for experienced ECIG users (N = 32) across conditions. In all other regards the figure is identical to Figure 4.

For the main effects of condition for the items "Craving" and "Urge," post-hoc analyses revealed that the magnitude of decrease in VAS scores was dependent on condition. Overall less pronounced VAS score reductions were observed for the 3 mg/ml+1.5Ω combination compared to others. For the item "Urge," mean VAS scores decreased significantly from baseline relative to the 10-puff bout and at the conclusion of the *ad libitum* bout for all conditions; however, reductions in scores were less pronounced for the 3 mg/ml+1.5Ω combination relative to other combinations. For example, immediately following the 10-puff bout, mean (SD) VAS score of 28.9 (4.4) observed for the 8 mg/ml+0.5Ω combination was significantly lower relative to the mean (SD) VAS score of 43.8 (4.1) for the 3 mg/ml+1.5Ω combination (Tukey's HSD, *p*s < .05). Following the conclusion of the *ad libitum* bout, the mean (SD) VAS score of 28.3 (4.8) observed for the 8 mg/ml+1.5Ω combination was significantly lower relative to the mean (SD) score of 39.0 (4.8) for the 3 mg/ml+1.5Ω combination (Tukey's HSD, *p*s < .05). For the item "Craving," no significant differences across conditions were observed in VAS score reduction following the 10-puff bout. At the conclusion of the *ad libitum* bout, more pronounced VAS score reductions for "Craving" were observed for the 3 mg/ml+0.5Ω and 8 mg/ml+1.5Ω combinations relative to the 3 mg/ml+1.5Ω combination (Tukey's HSD, *p*s < .05).

Questionnaire of Vaping Craving. As indicated in Table 3, no significant condition by time interactions were observed for Factors 1 (intention to vape) or 2 (anticipation of relief from vaping abstinence). However, significant main effects of time [*F*s (5, 155) ≥ 34.4, *p*s <.001] and main effects of condition [*F*s (3, 93) ≥ 3.8, *p*s <.05] were observed for both factors.

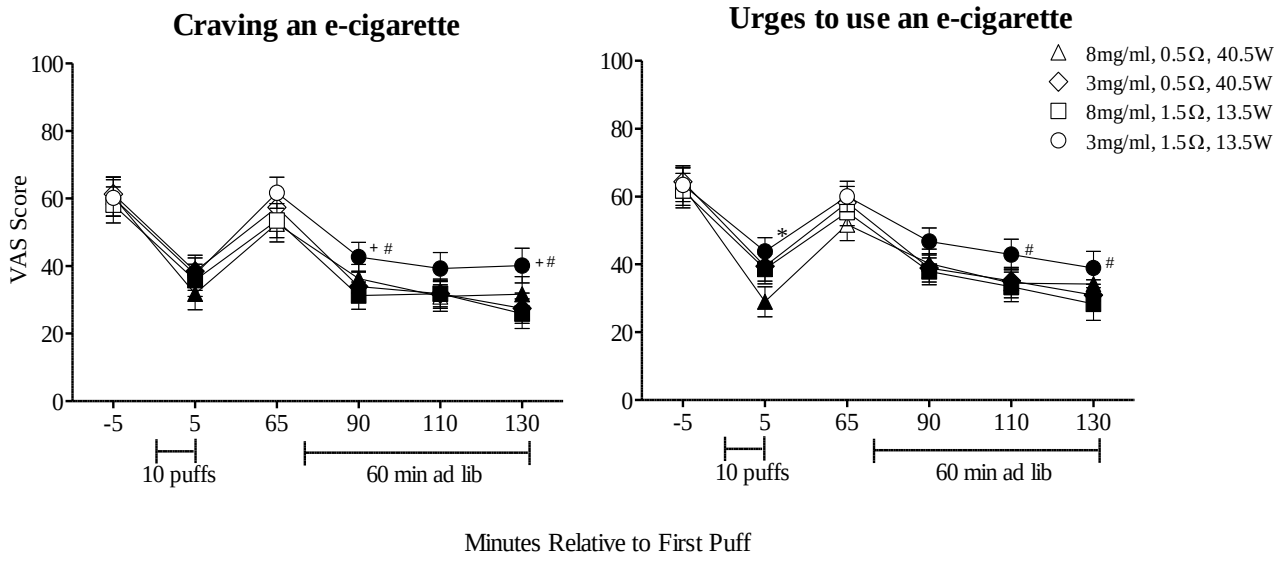

Figure 6. Mean (± SEM) ratings for the visual analog scale items "Craving an e-cigarette" (Left) and "Urges to use an e-cigarette" from the Hughes-Hatsukami withdrawal scale. In all other respects the figure is identical to Figure 4.

Post-hoc tests revealed that, scores for Factors 1 and 2 were reduced significantly from baseline following the 10-puff bout and *ad libitum* bout (Tukey's HSD, *p*s < .05). Collapsed across condition, mean (SD) score for Factor 2 was reduced significantly from a score of 17.3 (7.5) at baseline to a score of 11.1 (6.9) immediately after the 10-puff bout to a score of and 8.9 (7.0) at the conclusion of the *ad libitum* bout.

Also, post-hoc tests revealed that for both factors, the magnitude of reduction in scores was dependent on condition. Overall, for Factors 1 and 2, immediately following the 10-puff bout, the decrease in scores was significantly greater for the 8 mg/ml+0.5Ω combination relative to the 3 mg/ml+1.5Ω combination. For example, immediately following the 10-puff bout, the mean (SD) score for Factor 2 was 10.6 (7.6) for the 8 mg/ml+0.5Ω combination and was significantly lower relative to the mean scores of 16.0 (7.9) for the 3 mg/ml+1.5Ω combination (Tukey's HSD, ps < .05). No significant differences across conditions were observed for the *ad libitum* bout. A similar pattern was observed for Factor 1.

Direct Effects of Nicotine. No significant interactions of time and condition or main effects of condition were observed for any items from the Direct Effects of Nicotine Scale (Table 3). Significant main effects of time were observed for the items "Lightheaded" and "Nervous" [*F*s (5, 155) ≥ 7.7, *p*s <.01]. Overall, mean scores for "Lightheaded" increased significantly from baseline following the 10-puff bout for the 8 mg/ml+0.5Ω and 3 mg/ml+0.5Ω combinations, and not following the *ad libitum* bout (Tukey's HSD, *p*s < .05). Collapsed across these two combinations (the 8 mg/ml+0.5Ω and 3 mg/ml+0.5Ω), mean (SD) scores for "Lightheaded" increased from a score of 9.0 (15.7) at baseline to 22.1 (22.0) after the 10-puff bout and decreased to 10.5 (14.9) at the conclusion of the *ad libitum* bout. Post-hoc analyses revealed no significant differences for "Nervous" across time.

Direct Effects of ECIG Use. As indicated by Table 3, there was a significant time by condition interaction for the item "Did the e-cigarette make you dizzy" [F (12, 372) = 2.5, p <.01]. The interaction of time and condition is explained by significant differences in mean VAS scores for "Dizzy" across conditions immediately following the 10-puff bout and not during the *ad libitum* bout. Immediately following the 10-puff bout, the mean (SD) score for "Dizzy" was 36.2 (25.1) for the 8 mg/ml+0.5Ω combination and was significantly higher relative to the mean score of 17.9 (23.4) for the 3 mg/ml+1.5Ω combination and mean score of 24.5 (22.2) for the 8 mg/ml+1.5Ω combination (Tukey's HSD, $p < .05$). No significant differences in scores for "Dizzy" were observed across conditions during the *ad libitum* bout.

Figure 7 displays four items ("Dizzy," "Pleasant," "Right now" and "Satisfying"). Significant main effects of condition were observed for the items "Was the e-cigarette pleasant?," "Would you like another e-cigarette right now?," "Was the e-cigarette satisfying?," "Did the e-cigarette taste good?," "Did you like the smell of e-cigarette?," "Did the e-cigarette produce a visible cloud or vapor?" [Fs (3, 93) ≥ 2.8, ps <.05]. Because there was no true baseline timepoint for this subjective questionnaire (i.e., a timepoint prior to any product administration) no comparisons to baseline were possible. Post-hoc analyses revealed significant differences across conditions for the items "Pleasant," "Right now," "Satisfying," and "Taste good." For the item "Pleasant," generally scores were greater for the 3 mg/ml+0.5Ω combination and, at times, for the 3 mg/ml+1.5Ω combination relative to the 8 mg/ml+0.5Ω combination. For example, immediately following the 10-puff bout, mean (SD) VAS score of 64.2 (25.1) for the 3 mg/ml+0.5Ω combination and mean score of 62.8 (27.6) for the 3 mg/ml+1.5Ω combination were significantly higher relative to the mean (SD) score of 49.3 (31.5) for the 8 mg/ml+0.5Ω combination (Tukey's HSD, ps < .05). At the conclusion of the *ad libitum* bout, the mean (SD)

score of 67.6 (25.1) for the 3 mg/ml+0.5Ω combination was significantly higher relative to the score of 53.3 (27.1) for the 8 mg/ml+0.5Ω combination (Tukey's HSD, *p*s < .05).

For the item "Right now," scores were significantly lower for the 8 mg/ml+0.5Ω combination relative to the 3 mg/ml+1.5Ω combination immediately following the 10-puff bout and at 20 and 60 minutes of the *ad libitum* bout. Collapsed across time (following 10-puffs and during *ad libitum* use), mean (SD) VAS score for "Right now" was 43.0 (28.3) for the 8 mg/ml+0.5Ω combination and was lower relative to the score of 56.1 (28.5) for the 3 mg/ml+1.5Ω combination. For the item "Satisfying," following the 10-puff bout, mean (SD) scores were 69.3 (23.6) for the 3 mg/ml+0.5Ω combination and were significantly greater relative to mean scores of 57.6 (29.8) for the 8 mg/ml+0.5Ω combination and mean scores of 58.6 (27.5) for the 3 mg/ml+1.5Ω combination (Tukey's HSD *p*s < .05). Similarly, at the conclusion of the *ad libitum* bout, mean (SD) scores were 69.1 (23.6) for the 3 mg/ml+0.5Ω combination and were significantly greater relative to mean scores of 58.7 (28.7) for the 8 mg/ml+0.5Ω combination and scores of 55.6 (30.0) for the 3 mg/ml+1.5Ω combination (Tukey's HSD *p*s < .05). Finally, for the item "Taste good," greater scores were observed for the 3 mg/ml+0.5Ω combination relative to the 8 mg/ml+0.5Ω combination. For example, immediately following the 10-puff bout, mean (SD) scores for "Taste good" were 65.0 (23.0) for the 3 mg/ml+0.5Ω combination and were significantly higher relative to mean scores of 54.7 (30.8) for the 8 mg/ml+0.5Ω combination (Tukey's HSD *p*s < .05). Also, immediately following the 10-puff bout, mean (SD) scores were 62.8 (24.8) for the 3 mg/ml+1.5Ω combination and were significantly higher relative to mean scores of 52.8 (27.3) for the 8 mg/ml+1.5Ω combination (Tukey's HSD *p*s < .05).

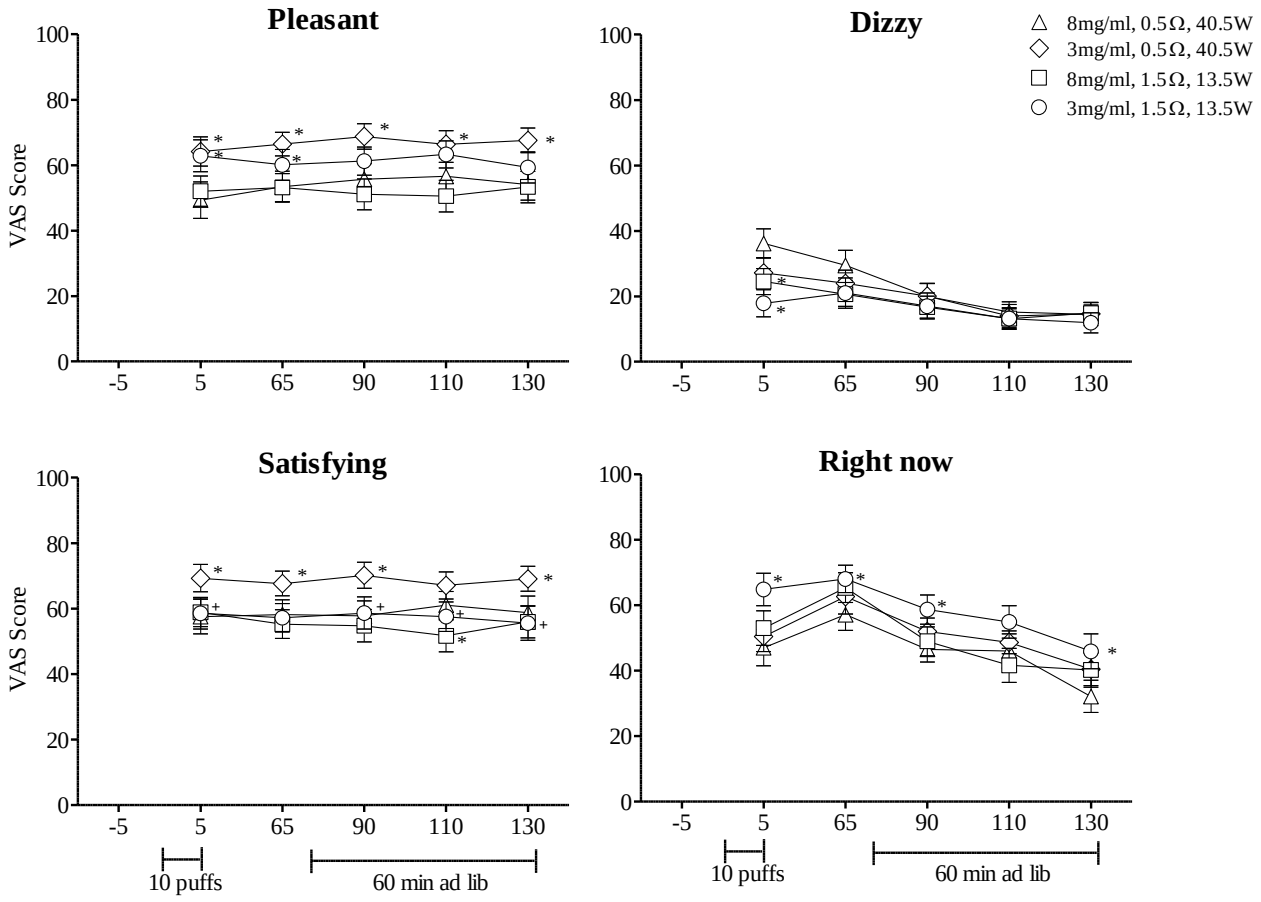

Figure 7. Mean ratings (+ SEM) for four visual analog scale items from the Direct Effects of ECIG use scale: "Was the e-cig pleasant?" (top left), "Did the e-cig make you feel dizzy?" (top right), "Was the e-cig Satisfying?" (bottom left), and "Would you like another e-cig right now?" (bottom right). In all other respects the figure is identical to Figure 4.

Immediately following the *ad libitum* bout, mean (SD) scores for "Taste Good" were 68.3 (22.4) for the 3 mg/ml+0.5Ω combination and were significantly higher relative to mean scores of 56.0 (30.0) for the 8 mg/ml+0.5Ω combination and mean scores of 58.2 (26.4) for the 3 mg/ml+1.5Ω combination (Tukey's HSD *p*s < .05).

Significant main effects of time were observed for the items "Did the e-cigarette make you feel more awake?," "Did the e-cigarette help calm you down?," "Did the e-cigarette help with concentration?," "Did the e-cigarette make you dizzy?," "Would you like another e-cigarette right now?," "How similar were the puffs you took to your own e-cigarette?" [*F*s (4, 124) ≥ 2.7, *p*s <.05]. Post-hoc analyses revealed significant differences across time for the items "Dizzy" and "Right now." The item "Dizzy," will not be describe in detail as a significant condition by time interaction for "Dizzy" was described in detail above. For the item "Right now," scores decreased over time, with some conditions demonstrating significant differences in reductions across the 10-puff bout and the conclusion of the *ad libitum* bout. For example, for the 3 mg/ml+1.5Ω combination immediately following the 10-puff bout, mean (SD) score for "Right now" was 64.8 (27.9) and decreased significantly to 45.8 (30.1) at the conclusion of the *ad libitum* bout (Tukey's HSD, *p*s < .05).

General Labeled Magnitude Scale. As indicated by Table 3, significant interactions of condition and time were observed for the items "Harshness/Irritancy" and "Throat Hit" [*F*s (3, 90) ≥ 2.8, *p*s <.05]. Post-hoc analyses revealed that for the item "Harshness/Irritancy," average scores were higher for the 8 mg/ml+0.5Ω and 8 mg/ml+1.5Ω combinations relative to the 3 mg/ml+0.5Ω and 3 mg/ml+1.5Ω combinations (see Figure 8). For example, immediately following the 10-puff bout, mean (SD) score of 59.9 (23.8) for the 8 mg/ml+0.5Ω combination was significantly higher relative to the mean (SD) score of 36.1 (17.9) for the 3 mg/ml+0.5Ω

combination and mean score of 35.8 (19.6) for the 3 mg/ml+1.5Ω combination (Tukey's HSD, *p*s < .05). Also following the 10-puff bout, the mean (SD) score of 55.5 (24.5) for the 8 mg/ml+1.5Ω combination was significantly higher relative to the mean (SD) score of 35.7 (19.6) observed for the 3 mg/ml+1.5Ω combination (Tukey's HSD, *p*s < .05). The same pattern was observed for the *ad libitum* bout.

Results for "Throat Hit" are illustrated in Figure 8. For the interaction between condition and time for "Throat Hit," post-hoc analyses revealed that immediately following the 10-puff bout, mean (SD) score of 70.2 (17.7) for the 8 mg/ml+0.5Ω combination was significantly higher relative to the mean (SD) score of 46.8 (18.1) for the 3 mg/ml+0.5Ω combination, the mean score of 58.7 (22.2) for the 8 mg/ml+1.5Ω combination, and the mean score of 36.22 (18.9) for the 3 mg/ml+1.5Ω combination (Tukey's HSD, *p*s < .05). Also, following the 10-puff bout, the mean (SD) score of 46.8 (18.1) for the 3 mg/ml+0.5Ω combination and the mean score of 58.7 (22.2) for the 8 mg/ml+1.5Ω combination were significantly higher relative to the mean score of 36.2 (18.9) for the 3 mg/ml+1.5Ω combination (Tukey's HSD, *p*s < .05). Similar patterns were observed after the *ad libitum* bout, though the differences in "Throat Hit" between the 3 mg/ml+0.5Ω and 3 mg/ml+1.5Ω combinations were no longer significant.

Significant main effects of condition were observed for the items "Flavor," "Harshness/Irritancy," "Throat Hit," and "Warmth" [*F*s (3, 90) ≥ 7.6, *p*s <.001]. For "Flavor," post-hoc tests revealed significantly higher scores, on average, for the 8 mg/ml+0.5Ω combination relative to the 8 mg/ml+1.5Ω and 3 mg/ml+1.5Ω combinations; significantly higher scores also were observed for 3 mg/ml+0.5Ω relative to the 3 mg/ml+1.5Ω combination (Tukey's HSD, *p*s < .05). Collapsed across time, mean (SD) score for "Flavor" was 52.9 (20.9) for the 8 mg/ml+0.5Ω combination and 54.3 (20.7) for the 3 mg/ml+0.5Ω combination and these

scores were significantly higher relative to the mean (SD) scores of 41.7 (19.0) for the 3 mg/ml+1.5Ω combination and 44.2 (20.7) for the 8 mg/ml+1.5Ω combination (Tukey's HSD, *p*s < .05). For the item "Warmth," post-hoc analyses revealed that, average scores for the 8 mg/ml+0.5Ω combination were significantly greater relative to the average scores observed for all other combinations following the 10-puff bout and *ad libitum* bout (Tukey's HSD, *p*s < .05). Immediately following the 10-puff bout, mean (SD) score of 52.4 (22.6) for the 8 mg/ml+0.5Ω combination was significantly greater relative to mean score of 45.2 (19.0) for the 3 mg/ml+0.5Ω combination, mean score of 41.7 (20.9) for the 8 mg/ml+1.5Ω combination, and mean score of 29.7 (16.2) for the 3 mg/ml+1.5Ω combination (Tukey's HSD, *p*s < .05). Following 10-puffs, scores for the item "Warmth" also were significantly greater for the 3 mg/ml+0.5Ω and 8 mg/ml+1.5Ω combination relative to the 3 mg/ml+1.5Ω combination (Tukey's HSD, *p*s < .05). Similar patterns were observed following the *ad libitum* bout.

Labeled Hedonic Scale. Significant interactions of condition and time were observed for the items "Harshness/Irritancy Liking" and "Throat Hit Liking" [*F*s (3, 90) ≥ 3.1, *p*s <.05] as were significant main effects of condition for "Harshness/Irritancy Liking" (Table 3). The interaction of condition and time for "Harshness/Irritancy Liking" is explained by significant differences in mean VAS scores across conditions immediately following the 10-puff bout and not following the *ad libitum* bout (see Figure 8). Immediately following the 10-puff bout, mean (SD) score for "Harshness/Irritancy Liking" was 43.3 (28.2) for the 8 mg/ml+0.5Ω combination and was significantly lower relative to the mean (SD) score of 54.5 (19.1) for the 3 mg/ml+0.5Ω combination, the mean score of 42.5 (23.5) for the 8 mg/ml+1.5Ω combination and the mean score of 54.5 (17.6) for the 3 mg/ml+1.5Ω combination (Tukey's HSD, *p*s < .05). Also, following the 10-puff bout, mean (SD) score for "Harshness/Irritancy Liking" for the 8

mg/ml+1.5Ω combination of 42.5 (23.5) was significantly lower relative to the mean score for the 3 mg/ml+1.5Ω combination of 54.5 (17.6; Tukey's HSD, *p*s < .05). Following the *ad libitum* bout, no significant differences in VAS scores were observed for across conditions for "Harshness/Irritancy Liking." Similarly, the interaction of condition and time for "Throat Hit Liking" is explained by significant differences in mean VAS scores across conditions immediately following the 10-puff directed bout and not following the *ad libitum* bout (see Figure 8). Following the 10-puff bout, mean (SD) score for "Throat Hit Liking" for the 8 mg/ml+0.5Ω combination was 47.3 (25.7) and was significantly lower relative to the mean score of 59.5 (21.4) for the 3 mg/ml+0.5Ω combination (Tukey's HSD, *p*s < .05).

Puff Topography

Mean (SD) puff duration, puff volume, flow rate, IPI, and puff number data for experienced ECIG users are displayed in Table 4. For puff duration, no significant time by condition interaction was observed, however, a significant main effect of condition [F (3, 90) = 29.3, $p < .001$] was observed. Post-hoc tests (Tukey's HSD) revealed that during the 10-puff bout and *ad libitum* bout, participants took significantly shorter duration puffs when using the 8 mg/ml+0.5Ω combination relative to all other combinations. For example, collapsed across time, mean (SD) puff durations of 2.2 sec (0.8) for the 8 mg/ml+0.5Ω combination were shorter relative to mean (SD) puff durations of 2.8 sec (0.9) for the 3 mg/ml+0.5Ω combination, mean puff durations of 3.3 sec (1.1) for the 8 mg/ml+1.5Ω combination, and mean puff durations of 3.9 sec (1.5) for the 3 mg/ml+1.5Ω combination (Tukey's HSD, *p*s < .05).

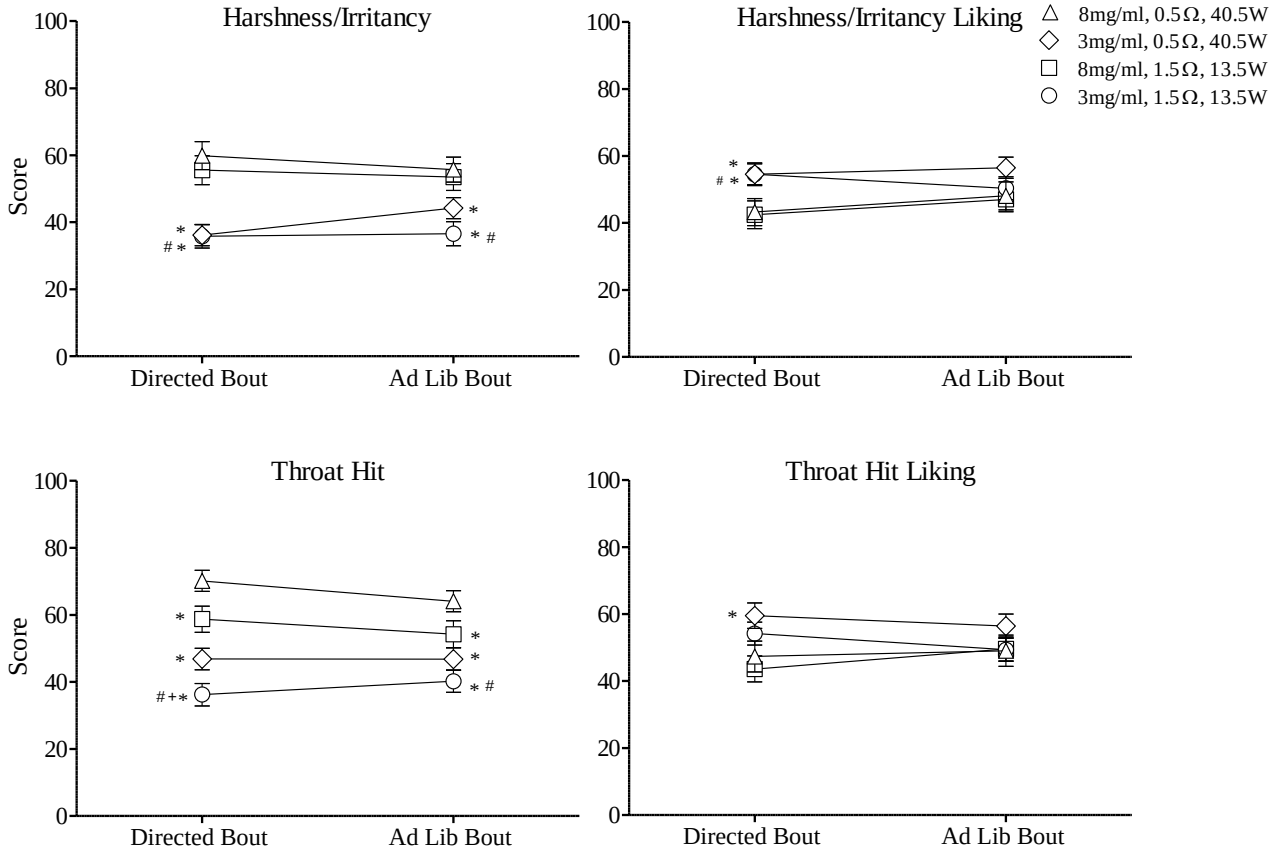

Figure 8. Mean ratings (+ SEM) for the items "Harshness/Irritancy" and "Throat Hit" from the general Labeled Magnitude scale (gLMS) and for the items "Harshness/Irritancy Liking" and "Throat Hit Liking" from the Labeled Hedonic Scale (LHS). Note that these items were administered on two occasions: immediately after the 10-puff directed bout and at the conclusion of the *ad libitum* bout. In all other respects the figure is identical to Figure 4.

Additional post-hoc testing revealed that, on average, participants took significantly longer duration puffs when using the 3 mg/ml+1.5Ω combination relative to the 3 mg/ml+0.5Ω combination and 8 mg/ml+1.5Ω combinations during the 10-puff bout and *ad libitum* bout. For example, during the 10-puff bout, mean (SD) puff duration of 4.0 sec (1.6) for the 3 mg/ml+1.5Ω combination was significantly longer relative to mean puff duration of 3.0 sec (1.0) for the 3 mg/ml+0.5Ω combination and mean puff duration of 3.3 sec (1.1) for the 8 mg/ml+1.5Ω combination (Tukey's HSD, *p*s < .05). No significant differences in puff duration were observed across the 10-puff bout and the *ad libitum* bout.

For puff volume, no significant time by condition interaction was observed. However, significant main effects of time [F (1, 30) = 5.3, p <.05] and condition [F (3, 90) = 9.8, p < .001] were observed. For the main effect of time, post-hoc testing revealed no significant differences for puff volume across the 10-puff bout and *ad libitum* bout for any study condition (Tukey's HSD). For the main effect of condition, post-hoc testing revealed that during the 10-puff bout, mean (SD) puff volume of 363.2 (147.3) for the 8 mg/ml+0.5Ω combination was significantly smaller relative to the mean (SD) puff volume of 519.6 (252.3) for the 3 mg/ml+0.5Ω combination and mean puff volume of 481.1 (275.4) for the 3 mg/ml+0.5Ω combination (Tukey's HSD, *p*s < .05). Also during the 10-puff bout, mean (SD) puff volume of 481.1 (275.4) for the 3 mg/ml+1.5Ω combination was significantly larger relative to mean puff volume of 384.4 (185.2) for the 8 mg/ml+1.5Ω combination (Tukey's HSD, *p*s < .05). During the *ad libitum* bout significantly larger puff volumes were observed for the 3 mg/ml+0.5Ω combination relative to the 8 mg/ml+0.5Ω combination (see Table 4).

For flow rate, no significant time by condition interaction was observed though a significant main effect of condition [F (3, 90) = 46.5, p < .001] was detected. Post-hoc tests revealed that during the 10-puff bout and *ad libitum* bout, significantly greater flow rate puffs were taken in the 3 mg/ml+0.5Ω combination relative to the 8 mg/ml+0.5Ω and 3 mg/ml+1.5Ω combinations; during both bouts flow rates for the 8 mg/ml+0.5Ω combination were significantly greater relative to the 8 mg/ml+1.5Ω and 3 mg/ml+1.5Ω combinations. For example, during the 10-puff bout mean (SD) flow rate of 176.2 ml/sec (60.1) for the 3 mg/ml+0.5Ω combination was significantly greater relative to mean flow rate of 160.2 ml/sec (55.4) for the 8 mg/ml+0.5Ω combination and a mean flow rate of 122.3 ml/sec (52.7; Tukey's HSD, ps < .05).

For IPI, a significant time by condition interaction was observed [F (3, 90) = 3.9, p <.05] in addition to significant main effects of time [F (1, 30) = 77.9, p <.001] and condition [F (3, 90) = 4.3, p < .05]. Because IPI was controlled experimentally during the 10-puff bout, differences in IPI across time likely are a result of this control. Indeed, post-hoc analyses revealed significantly longer IPI during the *ad libitum* bout relative to the 10-puff bout for all study conditions (Tukey's HSD, ps < .05). During the *ad libitum* bout, significantly longer IPIs were observed, on average, for the 8 mg/ml+0.5Ω combination relative to the 3 mg/ml+0.5Ω and 3 mg/ml+1.5Ω combinations. Mean (SD) IPI was 122.8 sec (88.6) for the 8 mg/ml+0.5Ω combination and was significantly longer relative to mean IPI of 85.5 sec (40.7) for the 3 mg/ml+0.5Ω combination and mean IPI of 81.0 sec (39.8) for the 3 mg/ml+1.5Ω combination (Tukey's HSD, ps < .05).

For puff number, a significant time by condition interaction was observed [F (3, 93) = 7.4, p <.001] as were significant main effects of time [F (1, 31) = 110.6, p <.001] and condition [F (3, 93) = 7.6, p <.001]. During the 10-puff bout, puff number was controlled experimentally resulting in significantly fewer number of puffs, on average, during the 10-puff bout relative to

the *ad libitum* bout across all conditions (Tukey's HSD, *p*s < .05). Also, post-hoc analyses revealed significant differences in puff number across conditions during the *ad libitum* bout. For example, mean (SD) puff number of 33.0 (16.4) for the 8 mg/ml+0.5Ω combination was significantly lower relative to mean puff number of 45.2 (17.1) for the 3 mg/ml+0.5Ω combination and mean puff number of 48.3 (29.7) for the 3 mg/ml+1.5Ω combination (Tukey's HSD, *p*s < .05). Also, during the *ad libitum* bout, significantly lower puff number was observed for the 8 mg/ml+1.5Ω combination relative to the 3 mg/ml+0.5Ω combination.

Amount of Liquid Consumed

The results for average amount of liquid consumed are displayed in Table 5. For liquid consumed, a significant time by condition interaction was observed [F (3, 93) = 13.7, p <.001] as were significant main effects of time [F (1, 31) = 74.2, p <.001] and condition [F (3, 93) = 26.9, p <.001]. Post-hoc analyses revealed significant differences in liquid consumption across the 10-puff bout and *ad libitum* bout with significantly greater liquid consumption observed for the 3 mg/ml+0.5Ω and 3 mg/ml+1.5Ω combinations during the *ad libitum* bout relative to the 10-puff bout (Tukey's HSD, *p*s < .05). For example, for the 3 mg/ml+0.5Ω combination, mean (SD) liquid consumption of 1.00 ml (0.67) during the *ad libitum* bout was significantly greater relative to the mean liquid consumption of 0.23 ml (0.19) during the 10-puff bout (Tukey's HSD, *p*s < .05). Post-hoc analyses also revealed differences in liquid consumption across condition during the 10-puff bout with greater liquid consumption for the 8 mg/ml+0.5Ω and 3 mg/ml+0.5Ω combinations relative to the 8 mg/ml+1.5Ω and 3 mg/ml+1.5Ω combinations. For example, during the 10-puff bout, mean (SD) liquid consumption for the 8 mg/ml+0.5Ω combination of 0.23 ml (0.33) was significantly greater relative to the mean liquid consumption of 0.07 (0.06) for the 8 mg/ml+1.5Ω combination and 0.11 (0.09) for the 3 mg/ml+1.5Ω combination; for the 3

mg/ml+0.5Ω combination, mean (SD) liquid consumption of 0.23 (0.19) was significantly greater relative to mean liquid consumption of 0.11 (0.9) for the 3 mg/ml+1.5Ω combination (Tukey's HSD, *p*s < .05).

During the *ad libitum* bout, liquid consumption was greater for the 3 mg/ml+0.5Ω and 3 mg/ml+1.5Ω combinations relative to all others. For example, mean (SD) liquid consumption for the 3 mg/ml+0.5Ω combination of 1.00 ml (0.67) was significantly greater relative to the mean liquid consumption of 0.50 ml (0.33) for the 8 mg/ml+0.5Ω combination and 0.60 ml (0.45) for the 3 mg/ml+1.5Ω combination; for the 3 mg/ml+1.5Ω combination, mean (SD) liquid consumption of 0.60 ml (0.45) was significantly greater relative to mean liquid consumption of 0.50 ml (0.33) for the 8 mg/ml+0.5Ω combination and 0.30 ml (0.22) for the 8 mg/ml+1.5Ω combination (Tukey's HSD, *p*s < .05).

Table 4.

Mean (SD) Puff Topography by ECIG Resistance and Liquid Combination for the Directed and Ad Libitum Bouts.

	ECIG Resistance and Liquid Combination			
	8 mg/ml_0.5Ω	**3 mg/ml_0.5Ω**	**8 mg/ml_1.5Ω**	**3 mg/ml_1.5Ω**
Puff Duration (sec)				
Directed	2.3 (0.8)	3.0 (1.0)*	3.3 (1.1)*	4.0 (1.6)*$^{+\#}$
Ad libitum	2.2 (0.9)	2.7 (0.7)*	3.3 (1.1)*	3.8 (1.5)*$^{+\#}$
Puff Volume (ml)				
Directed	363.2 (147.3)	519.6 (252.3)*	384.4 (185.2)	481.1 (275.4)*$^{\#}$
Ad libitum	331.2 (153.3)	463.9 (200.1)*	362.9 (175.8)	407.8 (204.9)
Flow Rate (ml/sec)				
Directed	160.2 (55.4)	176.2 (60.1)*	115.0 (42.2)*	122.3 (52.7)*$^{+}$
Ad libitum	154.6 (53.7)	176.6 (62.2)*	109.8 (36.7)*	109.7 (40.0)*$^{+}$
IPI (sec)				
Directed	27.7 (1.2)	27.2 (1.6)	25.8 (2.5)	25.8 (2.7)
Ad libitum	122.8 (88.6)	85.5 (40.7)*	107.1 (78.8)	81.0 (39.8)*
Puff Number				
Directed	10.0 (0.2)	10.1 (0.5)	10.0 (0.4)	10.0 (0.4)
Ad libitum	33.0 (16.4)	45.2 (17.1)*	35.4 (18.3)	48.3 (29.7)*$^{\#}$

Mean (SD) puff parameters for experienced ECIG users (N = 31) for a 10-puff directed ECIG use bout (30 sec IPI) and 60-minute *ad libitum* bout. A malfunction of the topography recording device resulted in incomplete data for one participant out of the 32 completers who were

included in all other analyses. Note, IPI (30 s) and puff number (10) were controlled experimentally during the 10-puff directed bout as described in the method and data are included here to demonstrate that control. Asterisks (*) indicate significant differences between the 8mg/ml+0.5Ω combination and all other combinations during that bout; crosses (+) indicate significant differences between the 3mg/ml+0.5Ω and 3mg/ml+1.5Ω combinations and pound symbols (#) indicate significant differences between the 3mg/ml+1.5Ω and 8mg/ml+1.5Ω combinations (Tukeys HSD; *p*s < .05).

Table 5.

Mean (SD) Liquid Consumed (in ml) by ECIG Liquid and Resistance Combination for the Directed and Ad Libitum Bouts.

	ECIG Resistance and Liquid Combination			
	8 mg/ml_0.5Ω	**3 mg/ml_0.5Ω**	**8 mg/ml_1.5Ω**	**3 mg/ml_1.5Ω**
Liquid consumed (ml)				
Directed	0.23 (0.19)	0.23 (0.19)	0.07 (0.06) *	0.11 (0.09) *$^{+}$
Ad libitum	0.50 (0.33)	1.00 (0.67) *	0.30 (0.22) *	0.60 (0.45) *$^{+\ \#}$

Mean (SD) liquid consumed (in milliliters) for experienced ECIG users (N = 32) following a 10-puff directed ECIG use bout (30 sec IPI) and 60-minute *ad libitum* bout. The total amount of liquid consumed across the 10-puff directed bout and *ad libitum* bout is provided for each condition. In all other respects, the table is identical to Table 4.

Discussion

Overview

ECIGs are an increasingly popular category of tobacco products that the U.S. FDA has the authority to regulate. ECIGs vary with regard to several user-modifiable device characteristics including device power (an interaction of heating coil resistance and battery voltage) and liquid constituents (e.g., flavorant, solvent, and nicotine concentration; Breland et al., 2017). Manipulating device characteristics and liquid constituents can influence ECIG acute effects (e.g., Hiler et al., 2017; Krishnan-Sarin, 2017; Rosbrook & Green, 2016; Spindle et al., 2018; Wagener et al., 2017), as can changes in user's behavior (Dawkins et al., 2016, 2018; Farsalinos et al., 2015; Hiler et al., 2017). While many ECIGs contain relatively high resistance coils ($\geq 1.5\Omega$), one category of ECIG includes "sub-Ohm" devices that use low resistance coils (e.g., $< 1\Omega$; Breland et al., 2017; Talih et al., 2017a). Relative to conventional ECIGs, "sub-Ohm" devices are capable of operating at higher power output (e.g., 50-300 W). Increasing ECIG power increases nicotine yield (Talih et al., 2015) and high power ECIGs deliver cigarette-like amounts of nicotine to users, even when paired with low liquid nicotine concentrations (e.g., 4 mg/ml; Wagener et al., 2017). However, the extent to which ECIG coil resistance influences nicotine delivery and user subjective effects had not been studied systematically until now. Further, no studies to date have characterized user puff topography when low resistance coils were used.

The aim of this clinical laboratory study was to examine, using a 2 x 2 within-subject design, the individual and combined influence of ECIG coil resistance and liquid nicotine concentration on nicotine delivery, HR, subjective experience, puff topography, and liquid consumption. Thirty-two experienced ECIG users completed four Latin-square ordered sessions that differed by ECIG coil resistance (0.5 Ω or 1.5 Ω) and liquid nicotine concentration (3 or 8

mg/ml) resulting in four study conditions: 8 mg/ml+0.5Ω, 3 mg/ml+0.5Ω, 8 mg/ml+1.5Ω, 3 mg/ml+0.5Ω. By manipulating ECIG coil resistance, device power varied across conditions: 40.5 W in the 0.5 Ω condition and 13.5 W in the 1.5 Ω condition. In each session, participants completed a 10-puff directed (30 sec IPI) and 60-minute *ad libitum* bout, with the two bouts separated by 60 minutes. The text below discusses the observed effects of these manipulations on plasma nicotine concentration, HR, subjective effects, puff topography, and liquid consumption. Regulatory implications are discussed and potential study limitations are described.

ECIG Acute Effects: Nicotine Delivery and HR

Nicotine delivery from ECIGs is related directly to ECIG coil resistance and liquid nicotine concentration, is physiologically active, and may depend on additional factors such as puff topography and user experience with devices containing low resistance coils. As illustrated by Figure 4, nicotine delivery was greatest for the 8 mg/ml+0.5Ω combination followed by the 3 mg/ml+0.5Ω combination, with the lowest nicotine delivery observed for the 3 mg/ml+1.5Ω combination. Overall, the study ECIG delivered higher concentrations of nicotine with the 0.5Ω coil resistance (40.5W) relative to the 1.5Ω coil resistance (13.5W), regardless of liquid nicotine concentration. However, within coil resistance condition, ECIGs containing higher liquid nicotine concentration (8 mg/ml) resulted in greater nicotine delivery to users. Because device power in the present study was manipulated by coil resistance, these results are consistent with pre-clinical laboratory studies that demonstrate that increasing ECIG power results in greater nicotine in the resulting aerosol (Talih et al., 2015). Furthermore, ECIG-associated nicotine delivery was physiologically active for all study conditions as indicated by increases in HR following ECIG-use. HR increased significantly from baseline immediately after the 10-puff bout and at 20, 40 and 60 minutes of the *ad libitum* bout (see Figure 5). Consistent with results

observed for blood plasma nicotine concentration, the most pronounced increases in HR were observed following use of the 0.5Ω coil resistance (40.5W) combinations. Altogether, these results suggest that decreasing coil resistance to increase overall ECIG power is one method for increasing nicotine delivery.

Similar to previous reports of experienced ECIG users (Dawkins et al., 2016; Hiler et al., 2017; Spindle et al., 2015, 2018; St Helen et al., 2016; Wagener et al., 2017), some individuals in the present study were able to achieve nicotine delivery that is commensurate with what is typically observed after smoking one tobacco cigarette (e.g., 15-20 ng/ml Patterson et al., 2003; Vansickel et al., 2010; Yan & D'Ruiz, 2015). Following the 10-puff bout, seven individuals obtained cigarette-like nicotine delivery (i.e., ≥15 ng/ml) when using the 8 mg/ml+ 0.5Ω combination (M = 23.2 ng/ml; SD = 7.1), three obtained cigarette-like nicotine delivery when using the 3 mg/ml+ 0.5Ω combination (M = 18.5 ng/ml; SD = 2.0) and three obtained cigarette-like delivery when using the 8 mg/ml+ 1.5Ω combination (M = 23.3 ng/ml; SD = 11.8); no participants obtained cigarette-like nicotine concentrations following 10 puffs from the 3 mg/ml+ 1.5Ω combination. Altogether, 18 experienced ECIG users obtained cigarette-like nicotine delivery (i.e., 15 ng/ml or greater) either following 10-puffs or during *ad libitum* use whereas 14 experienced ECIG users did not achieve cigarette-like nicotine delivery with any device/liquid combination at any timepoint. These results are consistent with previous reports (Dawkins & Corcoran, 2014) and demonstrate that there is considerable inter-individual variability for nicotine delivery from ECIGs, even when device and liquid characteristics are standardized across users.

The observed variability in ECIG-associated nicotine delivery across ECIG users, even when using the same ECIG device and liquid combination, may be explained by differences in

user puff topography. Previous clinical laboratory examinations of ECIGs indicate that experienced ECIG users obtain significantly more nicotine relative to ECIG-naïve cigarette smokers (when all relevant device and liquid features are held constant) because experienced users generally take longer/larger puffs (Farsalinos et al., 2015; Hiler et al., 2017). Notably, in the present study, the 18 participants who obtained cigarette-like plasma nicotine concentrations (≥ 15 ng/ml) in at least one condition took significantly longer and larger puffs, on average, relative to the 14 participants who did not achieve cigarette-like nicotine delivery in any study condition (see Table 6). For example, during the 10-puff bout, when using the 3 mg/ml+0.5Ω combination, those who obtained cigarette-like nicotine concentrations exhibited a mean (SD) puff duration of 3.4 sec (2.3) whereas those who failed to achieve cigarette-like nicotine delivery exhibited a mean (SD) puff duration 2.3 sec (0.7) puffs. Indeed, significant differences across these two groups were observed for puff duration and volume for every study condition and for the 10-puff bout and *ad libitum* bout. Interestingly, of the 18 ECIG users who obtained plasma nicotine concentrations ≥ 15 ng/ml in at least one condition, 16 reported prior experience with "sub-Ohm" ECIGs with low resistance coils. Therefore, most individuals who achieved cigarette-like nicotine delivery may have been able to so because they had previously learned the puff duration and puff volumes necessary to obtain high concentrations of nicotine from various ECIG models, including "sub-Ohm" devices, and adjusted their puffing parameters accordingly in this study. Altogether, these results suggest that puff duration and volume remain important variables for nicotine delivery, even in the case of low resistance/high power devices.

Table 6.

Mean (SD) Puff Duration and Volume by ECIG Resistance and Liquid Combination and by Nicotine Delivery Status for the Directed Bout.

	ECIG Resistance and Liquid Combination			
	8 mg/ml_0.5Ω	**3 mg/ml_0.5Ω**	**8 mg/ml_1.5Ω**	**3 mg/ml_1.5Ω**
Puff Duration (sec)				
≥ 15 ng/ml	2.6 (0.8)[a]	3.4 (0.9)[a]	3.8 (1.1)[a]	4.5 (1.8)[a]
< 15 ng/ml	1.9 (0.6)	2.3 (0.7)	2.7 (0.9)	3.3 (0.9)
Puff Volume (ml)				
≥ 15 ng/ml	415.7 (151.5)[a]	626.3 (254.3)[a]	468.8 (196.5)[a]	588.8 (324.0)[a]
< 15 ng/ml	295.7 (114.1)	382.4 (176.5)	267.6 (75.0)	342.6 (83.2)

Mean (SD) puff duration and volume for experienced ECIG users for a 10-puff directed ECIG use bout (30 sec IPI). Data are stratified by nicotine delivery status: experienced ECIG users who obtained or exceeded cigarette like plasma nicotine concentrations of 15 ng/ml (n = 18), in at least one study condition, and those who did not (n = 14). Symbols ([a]) indicate significant differences between groups for that condition (Independent t-tests; *p*s < .05).

Subjective Measures.

ECIG-associated abstinence symptom suppression, pleasurable ECIG-effects (e.g., satisfaction and pleasantness), ECIG-related sensory effects and the liking/disliking of sensory effects were measured in experienced ECIG users following a 10-puff ECIG use bout and following 60-minutes of *ad libitum* ECIG use. While all study conditions reduced abstinence symptoms, the 8 mg/ml+0.5Ω combination produced the most pronounced abstinence symptom suppression for some items. However, the highest ratings for subjective measures assessing satisfaction and taste were observed for the 3 mg/ml+0.5Ω combination. Finally, the magnitude of ECIG-related sensory effects (i.e., throat hit and harshness) was dependent on ECIG liquid nicotine concentration, and sometimes coil resistance.

Nicotine/tobacco abstinence symptoms, observed in ~ 12-hour abstinent experienced ECIG users, were attenuated following the use of each ECIG resistance/nicotine combination. Attenuation of nicotine/tobacco abstinence symptoms is demonstrated by reductions in symptom magnitude from baseline following ECIG use. Relative to baseline, scores for the Hughes-Hatsukami items “Anxious,” “Craving an e-cigarette/nicotine,” “Depression,” “Difficulty concentrating,” “Drowsy,” “Impatient,” “Irritable,” “Sweets” and “Urges to use an e-cigarette” decreased significantly following the 10-puff ECIG-use bout and 20, 40 and 60-minutes of the *ad libitum* bout. Similarly, significant decreases in scores from baseline were observed for the Questionnaire of Vaping Craving Factors 1 (intention to vape) and 2 (anticipation of relief from vaping abstinence) following 10-puffs and at 20, 40 and 60-minutes of the *ad libitum* bout.

While all study conditions reduced abstinence symptoms, the extent to which abstinence symptoms were suppressed was contingent on the ECIG coil resistance and liquid nicotine concentration combination. For some subjective items, more pronounced reductions were

observed for the 8 mg/ml+0.5Ω combination relative to the 3 mg/ml+1.5Ω combination. For example, immediately following the 10-puff bout, mean (SD) score for "Urges to use an e-cigarette" was significantly lower for the 8 mg/ml+0.5Ω combination relative to the 3 mg/ml+1.5Ω combination (see Figure 6). Similar patterns were observed for Factor 1 and 2 of the Questionnaire of Vaping Craving.

In addition to suppression of nicotine/tobacco abstinence symptoms, ECIG use also produced pleasurable subjective effects and the magnitude of these effects was dependent on ECIG coil resistance and liquid nicotine combination. Overall, the 3 mg/ml+0.5Ω combination was rated as more pleasant, satisfying, and better tasting than the 8 mg/ml+0.5Ω combination (see Figure 7). Similarly, the 3 mg/ml+1.5Ω combination was rated as more pleasant, and better tasting than the 8 mg/ml+0.5Ω combination. These results suggest a preference for the 3 mg/ml nicotine concentration, particularly when the 3 mg/ml concentration is paired with a low coil resistance (0.5Ω). As will be described below, preference for low liquid nicotine concentrations may be due to the differences in the sensory effects produced by low and high nicotine concentration liquids.

ECIG coil resistance and liquid nicotine concentration influenced several ECIG-related sensory effects and participants' liking/disliking of those sensory effects. Generally, participants reported that the 8 mg/ml+0.5Ω and 8 mg/ml+1.5Ω combinations produced significantly greater sensations of "Harshness/Irritancy" relative to the 3 mg/ml+0.5Ω and 3 mg/ml+1.5Ω combinations. Further, higher scores for "Harshness/Irritancy Liking" were observed for the 3 mg/ml+0.5Ω combination and the 3 mg/ml+1.5Ω combination relative to the 8 mg/ml+0.5Ω and 8 mg/ml+1.5Ω combinations. These results indicate that: 1) sensations of "Harshness/Irritancy" may be more contingent on liquid nicotine concentration rather than coil resistance and 2) the

lower sensations of “Harshness/Irritancy” produced by lower nicotine concentration liquids (i.e., 3 mg/ml) were preferable to users as indicated by higher scores for liking of “Harshness/Irritancy”. These results are consistent with several prior reports (Kim et al., 2016; Spindle et al., 2018) that similarly demonstrated that lower sensations of “Harshness/Irritancy” on the gLMS correspond with higher ratings of product liking, satisfaction, and pleasantness (Kim et al., 2016; Spindle et al., 2018). Notably, in the present study, significant positive correlations were observed for scores of pleasantness and ratings of liking for “Harshness/Irritancy” for the 3 mg/ml+0.5Ω combination (r = .78) and the 3 mg/ml+1.5Ω combination (r = .60; all ps < .01).

Sensations of “Throat Hit” were greatest for the 8 mg/ml+0.5Ω combination and lowest for the 3 mg/ml+1.5Ω combination. In general, “Throat Hit” sensations were higher when using the 0.5Ω coil resistance (40.5W) and when using the high nicotine concentration of 8 mg/ml. In other words, the extent to which high nicotine concentration liquids produce greater sensations of “Throat Hit” is dependent on coil resistance. Participants provided significantly higher scores for “Throat Hit Liking” for the 3 mg/ml+0.5Ω combination relative to the 8 mg/ml+0.5Ω combination, indicating preference for the lower “Throat Hit” sensations produced by the low liquid nicotine concentration. Similar to “Harshness/Irritancy” the lower “Throat Hit” ratings produced by the 3 mg/ml+0.5Ω combination coincided with higher ratings of pleasantness and satisfaction. Moreover, liking scores for “Throat Hit” were positively correlated with ratings of pleasant (r = .40; all p < .05).

Consistent with previous reports, results from the present study indicate that use of higher concentration nicotine liquids coincides with greater throat hit sensations (Etter, 2016). In the present study, throat hit sensations also were higher when using the study ECIG with 0.5Ω coil

resistance (40.5W). With regard to liking of throat hit sensations, the results observed here are consistent with previous clinical laboratory evaluations of ECIGs that indicate greater throat hit sensations are associated with lower ECIG satisfaction (Spindle et al., 2018) and liking (Goldensen et al., 2016). However, in survey studies of experienced ECIG users, strong throat hit is associated with greater product satisfaction (Etter, 2016; McQueen, Tower, & Sumner, 2011; Pokhrel, Herzog, Muranaka, & Fagan, 2015). These discrepant findings, between those observed in the clinical laboratory and in survey research, may be explained by the fact that throat hit is relative. That is, in laboratory studies participants may rate sensations of throat hit, from a given study ECIG, in comparison to the throat hit sensations provided by their own device and liquid. Conversely, in survey reports, participants' typically report on the throat hit from their own device and do not have another product as a comparator. Future studies seeking to evaluate sensory perceptions of a particular ECIG, may consider assessing sensations from a participants' own ECIG and liquid to compare differences in ECIG sensory effects.

Taken together, these subjective results have several important implications. Consistent with previous examinations, ~12-hour abstinent ECIG users exhibit nicotine/tobacco abstinence symptoms (e.g., anxiety, irritability, difficulty concentrating, craving) and these symptoms are attenuated reliably following ECIG use. The presence of physical and psychological symptoms following nicotine abstinence is one indicator of nicotine dependence and nicotine self-administration in an effort to relieve or alleviate these symptoms is another (DSM-IV, 2013). Similar to previous reports, the ECIG device and liquid combinations that delivered the greatest amounts of nicotine to users (i.e., 8 mg/ml+0.5Ω combination) also were accompanied by the most pronounced reductions in nicotine/tobacco abstinence symptoms (Dawkins et al., 2016, 2018; Hiler et al., 2017). Conversely, the 3 mg/ml+1.5Ω combination, that delivered the least

amount of nicotine to users, corresponded with the lowest reduction in abstinence symptoms following use. In addition to abstinence symptom suppression, pleasurable ECIG-effects were assessed and the highest ratings for pleasantness, satisfaction, taste, and liking of sensory effects of harshness/irritancy and throat hit were observed for the 3 mg/ml+0.5Ω combination. Conversely, the 8 mg/ml+Ω combination had the lowest ratings for pleasantness, satisfaction, taste, and liking of sensory effects. These results indicate that ECIG device and liquid combinations that deliver the most nicotine and suppress abstinence symptoms do not necessarily produce the most pleasurable/sensory effects. Importantly, these results suggest that factors aside from nicotine delivery and abstinence symptom suppression may reinforce ECIG use for nicotine-naïve individuals and those individuals who are nicotine/tobacco dependent. Consequently, policymakers seeking to reduce the reinforcing properties of ECIGs should account for factors such as pleasurable sensory stimuli, pleasantness, and taste when regulating these products.

Puff Topography

Several puff topography measures were assessed during a 10-puff ECIG use bout and during 60-minutes of *ad libitum* puffing including puff duration, puff volume, flow rate, IPI, and puff number. As indicated in Table 5, some puff topography variables differed significantly across study conditions. In general, longer duration puffs were taken by participants when using the lower nicotine concentration liquids of 3 mg/ml. In addition, longer duration puffs also were taken when using the study ECIG with 1.5Ω coil resistance (13.5W) relative to the 0.5Ω coil resistance (40.5W). Consistent with these results, the shortest duration puffs were observed for the 8 mg+0.5Ω combination whereas the longest duration puffs were observed for the 3 mg/ml+1.5Ω combination. Furthermore, participants took larger puff volumes when using the

lower nicotine concentrations (3 mg/ml) relative to higher nicotine concentrations (8 mg/ml), regardless of coil resistance. For example, during the 10-puff bout, average puff volume was significantly larger for the 3 mg/ml+0.5Ω combination relative to the 8 mg/ml+0.5Ω combination. A similar pattern was observed for the 1.5Ω resistance. Overall, the greatest puff volumes were observed for the 3 mg/ml+0.5Ω combination. During *ad libitum* puffing, participants increased puff frequency when using the two 3 mg/ml nicotine concentrations, regardless of coil resistance. For example, significantly more puffs were taken when using the 3 mg/ml+0.5Ω combination relative to the 8 mg/ml+0.5Ω combination. Similarly, significantly more puffs were taken when using the 3 mg/ml+1.5Ω combination relative to the 8 mg/ml+1.5Ω combination.

The puff topography results from the present study have several important implications. This study is the first to manipulate ECIG device settings and liquid nicotine concentration systematically and measure the puff topography of experienced ECIG users. Overall, experienced ECIG users increase puff durations when using an ECIG with 1.5Ω coil resistance (13.5W) relative to the 0.5Ω coil resistance (40.5W). Independent of liquid nicotine concentration, the study ECIG delivered nicotine less effectively with the 1.5Ω coil resistance (13.5W) relative to the 0.5Ω coil resistance (40.5W); therefore, increases in puff duration when using the 1.5Ω coil resistance (13.5W) ECIG may be in an attempt, by users, to obtain more nicotine. An alternate explanation is that participants took shorter puffs when using the 0.5Ω coil resistance (40.5W) in response to the large volumes of aerosol often generated by higher power devices (Etter, 2012; Gillman et al., 2016; Talih et al., 2017a). However, in the absence of an "own-brand" ECIG control and without objective measurement of the actual volumes of aerosol

generated and inhaled by participants, the reason for the observed changes in puff duration are unclear.

As has been observed in previous studies that varied liquid nicotine concentration (Dawkins et al., 2016, 2018; Hiler et al., 2017), experienced ECIG users in the present study took longer and larger puffs and increased puff frequency when using the study ECIG containing 3 mg/ml nicotine concentration relative to the 8 mg/ml nicotine concentration. The longer/larger and more frequent puffs may have been in an attempt, by experienced users, to obtain more nicotine from the low concentration nicotine liquid (3 mg/ml). Indeed, the 3 mg/ml+1.5Ω combination delivered the least amount of nicotine to users and produced the least pronounced abstinence symptom suppression. Alternatively, the shorter duration puffs observed for the 8 mg/ml+0.5Ω combination may have been a response to the upper airway sensations produced by this ECIG combination. The 8 mg/ml+0.5Ω combination was rated as producing the most harshness/irritancy and throat hit sensations and had the lowest scores for liking on both of these measures.

Amount of Liquid Consumed

As indicated in Table 5, liquid consumption during the 10-puff ECIG bout and the 60-minute *ad libitum* bout differed by ECIG coil resistance and liquid nicotine concentration. Under controlled puffing conditions (i.e., 10-puffs), participants consumed almost twice as much liquid when using the ECIG with the 0.5Ω coil resistance (40.5W) ECIG relative to the 1.5Ω coil resistance (13.5W), independent of liquid nicotine concentration. For example, following 10-puffs, mean (SD) liquid consumed was 0.23 ml (0.19) for the 3 mg/ml+0.5Ω combination and was 0.11 ml (0.09) for the 3 mg/ml+1.5Ω combination. These differences in liquid consumption resulted in significantly higher average plasma nicotine concentration for the 3 mg/ml+0.5Ω

combination (M = 7.0 ng/ml; SD = 5.0) relative to the 3 mg/ml+1.5Ω combination (M = 4.6 ng/ml; SD = 3.3). A similar pattern for liquid consumption and nicotine delivery was observed for the 8 mg/ml+0.5Ω combination and the 8 mg/ml+1.5Ω combination, with the greater liquid consumption for the 8 mg/ml+0.5Ω combination corresponding with higher plasma nicotine concentrations.

At the conclusion of *ad libitum* puffing, the greatest amount of liquid had been consumed when using the 3 mg/ml+0.5Ω combination. Participants consumed almost twice as much liquid, on average, when using the 3 mg/ml+0.5Ω combination (M = 1.0 ml; SD = 0.7) relative to the 8 mg/ml+0.5Ω combination (M = 0.5 ml; SD = 0.3) in order to achieve comparable plasma nicotine concentrations. These results have several important implications. When puff number is held constant, the ECIG with the 0.5Ω coil resistance (40.5W) resulted in greater liquid consumption relative to the ECIG with the 1.5Ω coil resistance (13.5W). During *ad libitum* use, users take longer, larger and more frequent puffs and consume greater amounts of liquid when the 0.5Ω coil resistance (40.5W) is paired with low concentration nicotine liquid (i.e., 3 ng/ml), perhaps in an effort to increase nicotine delivery. These results are consistent with a recent study in which experienced ECIG users (N = 20) used each of the following ECIG configurations *ad libitum* over the course of one week: an ECIG containing low nicotine concentration (6 mg/ml) and fixed device power, low nicotine and adjustable power, high nicotine (18 mg/ml) and fixed device power, and high nicotine and adjustable device power (Dawkins et al., 2018). Experienced ECIG users increased puff duration, puff number and consumed more ECIG liquid daily when using the low liquid nicotine concentration (Dawkins et al., 2018). Also, when permitted, users increased device power to a greater extent when the ECIG contained a low nicotine concentration (Dawkins et al., 2018). Notably, use of the ECIG with low nicotine and

adjustable power configuration exposed users to greater amounts of formaldehyde (Dawkins et al., 2018). In addition, pre-clinical laboratory studies indicate that relative to the aerosols generated from low power ECIGs, the aerosols produced by high power ECIGs contain greater amounts of non-nicotine toxicants (El-Hellani et al., 2016; Gillman et al., 2016). Taken together, these results indicate that when high power ECIGs (i.e., 0.5Ω coil resistance, 40.5W) are paired with low nicotine concentrations, users may increase puff duration, volume, frequency, and inhale more ECIG liquid, possibly exposing themselves to greater amounts of non-nicotine toxicants contained in ECIG aerosols (Geiss, Bianchi, & Barrero-Moreno, 2016). Because the present study did not examine toxicant exposure, more work will be needed to elucidate actual toxicant exposure from the ECIG device/liquid configuration and puffing parameters observed here.

Regulatory Implications

To protect public health, FDA has stated its intention to regulate ECIGs and their "parts and components" under the "deeming" statute of The Family Smoking Prevention and Tobacco Control Act. Manufacturers and retailers of ECIGs must obtain authorization from FDA in order to market their products in the U.S. To obtain authorization, premarket applications must be submitted to FDA by 2022 demonstrating that a given ECIG meets public health standards. Adequate evaluation of these applications will necessitate empirical evidence describing what ECIGs do, what ingredients are contained in their liquids, what toxicants are contained in their aerosols, and the extent to which ECIGs will have a positive or negative impact on public health. One public health concern regarding ECIGs is their potential to promote or support nicotine dependence. Understanding the dependence potential of ECIGs will require an understanding of the factors that influence nicotine delivery and other acute effects (i.e., suppression of abstinence

symptoms and pleasurable effects following use; Carter et al., 2009; Eissenberg, 2004; Glautier, 2004). The available empirical evidence demonstrates that liquid nicotine concentration, solvent ratios such as PG:VG, and user puffing behavior influence ECIG nicotine delivery and acute effects (Dawkins et al., 2016, 2018; Farsalinos et al., 2015; Hiler et al., 2017; Spindle et al., 2018). However, because ECIG coil resistance and liquid nicotine concentration have not been manipulated systematically, the influence of these factors on ECIG acute effects remain unclear.

Despite remaining questions regarding ECIG nicotine delivery, some jurisdictions already regulate ECIG device and liquid characteristics. The European Union's Directive 2014/40/EU limits liquid nicotine concentrations of ECIG liquids to ≤ 20 mg/ml. The intention of the directive is to constrain nicotine delivery such that the nicotine delivery from ECIGs does not exceed the nicotine delivery from a tobacco cigarette (~15 ng/ml; Vansickel et al., 2010; (http://ec.europa.eu/health/tobacco/products_en). As is evident, the directive considers one factor that influences ECIG-associated nicotine delivery (i.e., liquid nicotine concentration) and fails to regulate several other factors such as device characteristics, liquid solvents such as PG: VG, and user puff topography. Consequently, users can defeat the intent of the directive by manipulating device and/or liquid characteristics and/or by changing their puffing behavior. As demonstrated by results from the present study, some experienced ECIG users can meet or exceed the nicotine delivery profile of smoking a single tobacco cigarette when coil resistance is low (0.5Ω)/power is high (40.5W) even when liquid nicotine concentration is far below EU's limit of ≤ 20 mg/ml. Results from this study and others (Dawkins et al., 2016) also indicate that when liquid nicotine concentration is low, users take longer/larger puffs, increase puff frequency and consume more ECIG liquid, perhaps in an effort to achieve cigarette-like nicotine delivery. Therefore, regulations like the European Union's Directive 2014/40/EU, that fail to account for device

characteristics and user puff topography, may result in greater ECIG liquid consumption and inhalation of more ECIG aerosol as users seek desired effects from ECIGs.

Results from the present study, and others, should serve to inform regulators that regulation of any one factor that influences ECIG nicotine delivery (e.g., liquid nicotine concentration) may have limited utility. As indicated in the present study, should regulators restrict liquid nicotine concentration in isolation (as has been done by EU), such a regulation may prompt users to increase device power (either by reducing coil resistance or increasing battery voltage) and increase puff volume, duration, and frequency to achieve desired nicotine delivery. Therefore, effective policies aimed at limiting ECIG nicotine delivery will need account for all relevant factors including ECIG liquid constituents (nicotine concentration, and solvent ratio), device power (including the factors that influence power: coil resistance and voltage), and user puff topography. One way regulate the amount of nicotine delivered from ECIGs in a manner that accounts for all factors relevant to ECIG nicotine delivery simultaneously is to constrain nicotine flux (Shihadeh & Eissenberg, 2015). Nicotine flux, defined as the amount of nicotine emitted from an ECIG per unit of time, is proposed as a regulatory target for regulating ECIG nicotine delivery (Shihadeh & Eissenberg, 2015). Nicotine flux can be predicted through an available mathematical model that accounts for the variability in several factors known to influence ECIG nicotine emissions and delivery including device power, liquid nicotine concertation, solvent ratio, and user puffing behavior (Talih et al., 2017). Product standards derived using the nicotine flux model would allow ECIG variables known to influence ECIG nicotine emissions to vary so long as nicotine flux stays within an allowable range. The allowable flux range may be high enough to allow ECIGs to deliver enough nicotine to suppress nicotine/tobacco abstinence symptoms in nicotine dependent cigarette smokers but

not so high as to promote and facilitate nicotine dependence in nicotine-naïve youth and young adults (Shihadeh & Eissenberg, 2015). In addition, the nicotine flux model could also be used to identify ECIG product parameters (e.g., allowable device power) that allow ECIGs to emit given amounts of nicotine to users while restricting other toxicant emissions (i.e., carbonyl compounds) produced by ECIGs. In sum, to regulate ECIG nicotine emissions effectively, policymakers will need to identify a method that can account for the variability in ECIG device and liquid characteristics as well as user behavior. Nicotine flux currently is one proposed regulatory framework to account for all factors that influence ECIG nicotine emissions.

Limitations

This study had several notable limitations that should be considered. In the present study, puff duration and volume were not controlled during the 10-puff directed bout. As a result, significant differences in puff duration and puff volume emerged across study conditions. Overall, participants took shorter and smaller puffs when using the 8 mg/ml+0.5Ω combination relative to other combinations. Given that longer and larger puffs are associated with greater nicotine yield (Talih et al., 2015), the shorter and smaller puffs exhibited during use of the 8 mg/ml+0.5Ω combination may have resulted in less pronounced nicotine delivery for this study combination. Future studies seeking to parse the influence of ECIG device and liquid characteristics on ECIG acute effects may consider even more controlled puffing regimens that standardize puff duration and volume (e.g., Zacny & Stitzer, 1988). However, given that this study was the first to characterize puff topography during use of a low resistance "sub-Ohm" device, identification the appropriate puff durations and volumes prior to the start of the study would have been challenging. Further, arbitrarily selecting and asking participants to take certain puff durations and volumes may have been aversive to some participants, particularly for those

resistance/nicotine combinations (i.e., 8 mg/ml+0.5Ω combination) that participants rated as being more harsh and irritating than others. Second, while all 32 participants who completed the study were experienced ECIG users, 14 reported no prior experience with low resistance ECIGs. Differential experience with some ECIG device settings may have influenced puff topography and thereby nicotine delivery from those individuals. When feasible, future studies seeking to characterize nicotine delivery and puff topography of a particular device may consider first to recruit only those individuals who are experienced and capable of obtaining nicotine from that device.

Another limitation involves the absence of a study condition that includes ECIG users' own ECIG device and liquid. Investigators may consider including such a condition as a comparator for nicotine delivery and abstinence symptom suppression. In the case of the present study, the primary purpose was to evaluate device and liquid characteristics in a systematic manner and ECIG user's own devices and liquids would vary greatly. Moreover, several recent studies have characterized nicotine delivery and abstinence symptom suppression of experienced ECIG users following 10-puffs and/or *ad libitum* use of their own ECIG device and liquid (Spindle et al., 2015, 2017; Wagener et al., 2017). Nevertheless, an own brand ECIG comparison may have provided context and explanation of some results obtained in the present study (i.e., sensory effects).

Conclusions

This within-subject, clinical laboratory study examined the individual and combined influence of ECIG coil resistance and liquid nicotine concentration on nicotine delivery, subjective effects, HR, puff topography, and liquid consumption. Overall, results indicate that greater nicotine delivery was observed for the 0.5Ω coil resistance (40.5W) relative to the 1.5Ω

coil resistance (13.5W), with the highest nicotine delivery observed after use of the 8 mg/ml+0.5Ω combination. Abstinence symptoms were suppressed across all study conditions but were most pronounced following use of the 8 mg+0.5Ω combination. However, pleasurable aspects of ECIG use and sensory effects were rated more favorably for the 3 mg+0.5Ω combination relative to the 8 mg+0.5Ω combination. Use of the ECIGs containing low liquid nicotine concentration of 3 mg/ml resulted in longer/larger puffs, and increased puff frequency. As anticipated, following 10 puffs, the greatest amount of liquid was consumed when using the ECIG with 0.5Ω coil resistance (40.5W). However, during *ad libitum* use, the greatest amounts of liquid were consumed when using the ECIGs containing 3 mg/ml nicotine concentration. In light of results from the present study, and previous studies evaluating ECIG acute effects, regulators will need to account for several factors including ECIG device characteristics, liquid constituents, and user puffing behavior when making regulatory decisions intended to control ECIG effects.

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APPENDIX A

Hughes & Hatsukami Tobacco Withdrawal Symptom Questionnaire

These phrases may or may not describe how you feel right now. Please respond to each word of phrase with how you feel RIGHT NOW

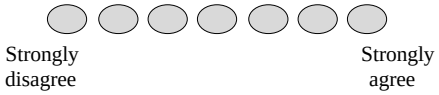
	Not at All	Extremely
1. Urges to use an e-cigarette		
2. Irritability/frustration/anger		
3. Anxious		
4. Difficulty Concentrating		
5. Restlessness		
6. Hunger		
7. Impatient		
8. CRAVING an e-cigarette		
9. Drowsiness		
10. Depression/ feeling blue		
11. Desire for Sweets		

APPENDIX B

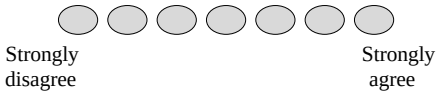
Questionnaire of Vaping Craving

For each item, please indicate how you feel RIGHT NOW

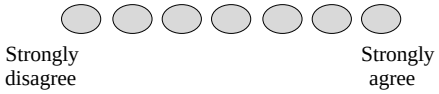
I have a strong desire for an e-cig right now.



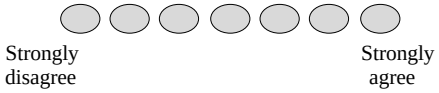
I have an urge for an e-cigarette.



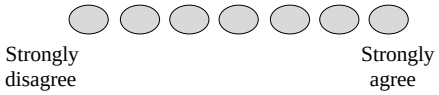
All I want right now is an e-cigarette.



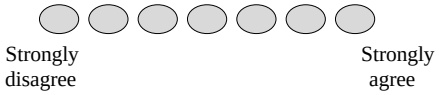
I am missing vaping right now.



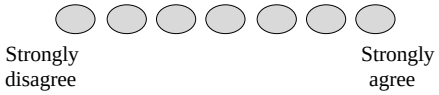
I am craving an e-cigarette right now.



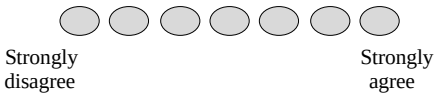
I need to vape now.



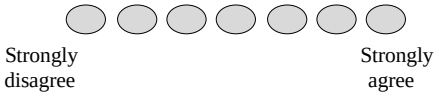
I am going to vape as soon as possible.



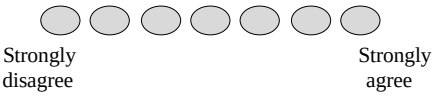
I will vape as soon as I get the chance.



Nothing would be better than vaping right now.



Vaping would make me happier now.



APPENDIX C

Direct Effects of Nicotine Scale (Modified)

These phrases may or may not describe how you feel right now. Please respond to each word or phrase with how you feel RIGHT NOW by drawing a vertical mark anywhere along the horizontal line.

	Not at all	**Extremely**
1. Nauseous		
2. Lightheaded		
3. Nervous		
4. Sweaty		
5. Headache		
6. Excessive salivation		
7. Heart pounding		
8. Confused		
9. Weak		

APPENDIX D

Direct Effects of ECIG Use Scale (Modified)

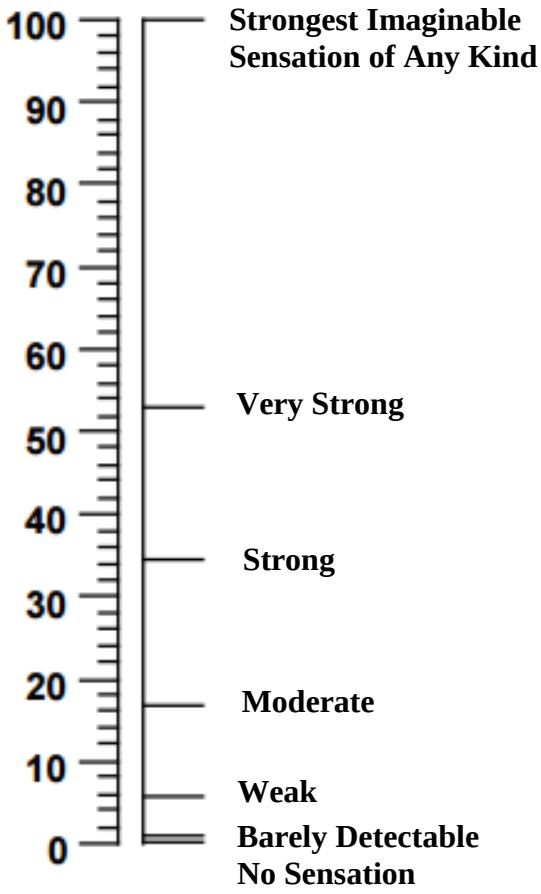
These phrases may or may not describe how you feel right now. Please respond to each word or phrase with how you feel RIGHT NOW by drawing a vertical mark anywhere along the horizontal line.

	Not at all — **Extremely**
1. Was the e-cig satisfying?	
2. Was the e-cig pleasant?	
3. Did the e-cig taste good?	
4. Did the e-cig make you dizzy?	
5. Did the e-cig calm you down?	
6. Did the e-cig help you concentrate?	
7. Did the e-cig make you feel more awake?	
8. Did the e-cig reduce your hunger for food?	
9. Did the e-cig make you sick?	
10. Would you like to use another e-cig RIGHT NOW?	
11. Did you enjoy the smell of the e-cig?	
12. How similar were the puffs to your own e-cig?	
13. Did the e-cig produce visible vapor or cloud?	
14. How important was the presence of the vapor or cloud to you?	

APPENDIX E
General Labeled Magnitude Scale

For each item, please indicate how you would describe the ECIG you just used by placing a mark on the vertical numbered line.

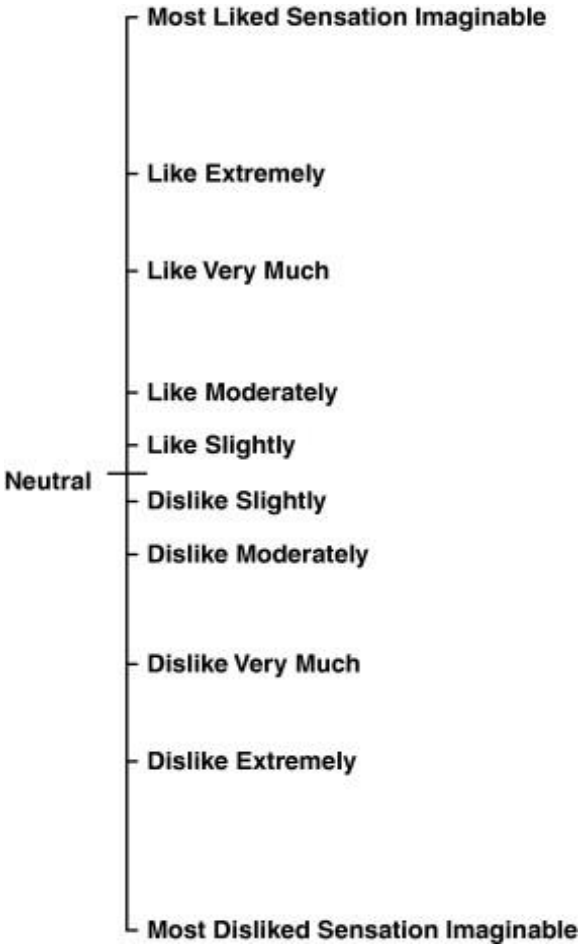
How would you describe the overall flavor sensation of the ECIG you just used?



APPENDIX F
Labeled Hedonic Scale

For each item, please indicate how you would describe the ECIG you just used by placing a mark on the vertical line.

How would you describe the overall flavor sensation of the ECIG you just used?



Vita

Marzena M. Hiler was born in Krakow, Poland on December 8, 1989. She is a graduate of Canyon high school in Anaheim, CA and has a B.A. in Psychology and Social Behavior from the University of California Irvine in Irvine, CA, which she received in 2012. She also received her M.S. in psychology from Virginia Commonwealth University (VCU) in 2016. She began the Health psychology doctoral program at VCU in August, 2014.