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Physiological and Subjective Effects of Protonated Nicotine Liquids in Tobacco Users

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PHYSIOLOGICAL AND SUBJECTIVE EFFECTS OF PROTONATED NICOTINE LIQUIDS IN TOBACCO USERS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

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List of Abbreviations

ANOVA	analysis of variance
BP	blood pressure
BPM	beats per minute
CO	carbon monoxide
CSTP	Center for the Study of Tobacco Products
ECIG	electronic cigarette
gLMS	General Labeled Magnitude Scale
HR	heart rate
IRB	Institutional Review Board
IPI	Inter-puff interval
LOQ	limit of quantification
min	minute(s)
mg	milligram
ng	nanogram
ml	milliliter
NRT	nicotine replacement therapy
PG	propylene glycol
ppm	concentration in parts per million
VAS	visual analog scale
VG	vegetable glycerin

Abstract

PHYSIOLOGICAL AND SUBJECTIVE EFFECTS OF PROTONATED NICOTINE LIQUIDS IN TOBACCO USERS.

By Alisha N. Eversole, B.A.

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Electronic cigarettes (ECIGs) produce an aerosol by heating a liquid that often contains nicotine. The nicotine can be protonated, potentially making the aerosol easier to inhale than freebase nicotine. This study's purpose was to determine, in inhaled tobacco product users, the effects of three concentrations of protonated nicotine aerosolized at two different power settings (in watts).

Twenty-two participants completed six sessions that varied by liquid nicotine concentration (10, 15, or 30 mg/ml protonated nicotine) and device power (15 or 30 W). Participants took 10 puffs from each product and then used each product for 60 minutes *ad libitum*. Plasma nicotine concentration, puff topography, and subjective effects were measured.

Findings from the present study suggest that liquid protonated nicotine concentration and device power setting influence ECIG nicotine delivery, user behavior, and subjective effects associated with use of ECIG devices containing protonated nicotine. For example, increases in one or more than one of these factors leads to increases in plasma nicotine concentration. This effect emphasizes the need to consider several factors in order to effectively regulate the nicotine delivery of ECIGs.

Physiological and Subjective Effects of Protonated Nicotine Liquids in Tobacco Users.

Tobacco smoking is the leading cause of preventable death worldwide, and in the United States an estimated one in five deaths is attributed to cigarette smoking each year (USDHHS, 2014). Life expectancy of cigarette smokers is more than ten years shorter than individuals who never smoked (Jha et al., 2013). Eight million people worldwide die each year from tobacco-related diseases (World Health Organization, 2019). Combustible cigarette smoking causes many illnesses including cancer, heart disease, stroke, lung diseases, chronic obstructive pulmonary disease, and diabetes (USDHHS 2014). Cigarette smokers also are at increased risk for tuberculosis, pneumonia, and impaired immune function (USDHHS, 2014). In the United States, more than 16 million people live with a tobacco-related disease (USDHHS 2014). The estimated economic toll of tobacco-related death and disease in the United States is more than $300 billion (USDHHS, 2014; Xu et al., 2015). Despite the well-documented deleterious health effects of smoking, 13.7% of adults, 5.8% of high-school students, and 2.3% of middle-school students are current (i.e., past 30-day) cigarette smokers (Creamer et al., 2019; Wang et al., 2019). Efforts aimed at reducing tobacco smoking and exposure to tobacco smoke are of utmost importance to the quality of life of the American population and to the American economy.

Tobacco smoke contains over 2,500 chemical constituents, including many known carcinogens (CDC, 2010) that can be found in mainstream and secondhand tobacco smoke (Öberg et al., 2011). Inhalation of tobacco smoke directly exposes the user to carcinogenic chemicals and other toxicants and primarily is responsible for the well-documented increases in death and disease that occur in tobacco smokers. The negative health effects of exposure to these toxicants have been established (Buran & Samet, 2020; CDC, 2010; Öberg et al., 2011). The

detrimental health effects of tobacco products are among the primary motivators for efforts aimed at tobacco use prevention (especially among youth) and cessation.

In 2015, 68% of combustible tobacco cigarette smokers reported that they want to stop smoking completely, and 55.4% attempted to quit (Babb et al., 2017; USDHHS, 2020). However, only 7.4% of tobacco smokers successfully quit in 2017 (USDHHS, 2020). Nicotine, a constituent of tobacco and a psychomotor stimulant drug, produces dependence in a majority of users and makes smoking cessation difficult (Prochaska & Benowitz, 2019), in part due to aversive abstinence symptoms (e.g., craving, irritability, insomnia) that accompany abrupt smoking cessation (Hughes & Hatsukami, 1986; Robinson et al., 2019). Nicotine binds as an agonist at nicotinic acetylcholine receptors, and this action in the brain primarily is responsible for the drug's psychoactive and dependence-producing effects (Benowitz, 2010; Leonard & Bertrand, 2001). Neuroadaptive changes occur in response to repeated nicotine exposure; after abrupt smoking/nicotine cessation, this neuroadaptation causes decreased activation of the dopaminergic system, leading to aversive abstinence symptoms (Benowitz, 2010; Prochaska & Benowitz, 2019). One recommended first-line treatment of tobacco dependence is counseling combined with nicotine replacement therapy (NRT; i.e., nicotine patch, nicotine gum) (Lindson et al., 2019). The administration of medicinal nicotine is safer than administration of nicotine via combustible cigarettes and helps to prevent relapse to smoking by alleviating aversive nicotine/tobacco abstinence symptoms (Germovsek et al., 2020; Wadgave & Nagesh, 2016) and blunting the effects of a concurrently administered cigarette (Foulds et al., 1992). Clinical trials have established this combination as the most effective intervention leading to cessation (Hartmann-Boyce et al., 2018a), and it can produce a significant increase in the likelihood of complete and sustained smoking cessation (Cahill et al., 2013; Hartmann-Boyce, et al., 2018a).

Though the increase in likelihood of successful cessation is statistically significant, approximately 7% of smokers who use the recommended medicinal cessation aids successfully quit using tobacco products (Hartmann-Boyce et al., 2018a). Due to the small proportion of smokers who successfully quit using NRT and other pharmacologic smoking cessation aids, the search for more effective treatment options persists.

In 2006, a new tobacco product entered the United States marketplace: electronic cigarettes (ECIGs). ECIGs have been studied as a potential cessation aid and/or a reduced harm product, based on the ability of some of these products to deliver nicotine with fewer carcinogenic chemicals compared to cigarettes (Gentry et al., 2019; St. Helen et al., 2020). Additionally, public health concerns have been raised regarding ECIG use as, at least in the US, approximately 23% of previously nicotine-naïve youth and young adults (age 14-30) have initiated tobacco use with ECIGs (Soneji et al., 2017). As highlighted below, youth ECIG initiation is a public health risk because ECIGs are capable of delivering nicotine to blood and brain (Voos et al., 2019), and nicotine exposure can harm the developing brain and cause dependence (England et al., 2015; NASEM, 2018). Also, the risk profile associated with long-term ECIG use is uncertain (Callahan-Lyon, 2014; Franck et al., 2016), and youth who initiate tobacco use with ECIGs are approximately three times more likely to initiate cigarette smoking relative to youth who do not use ECIGs (Soneji et al., 2017). The sections below provide an overview of ECIGs as a tobacco product class, and then describe the ways they may be used, the influence of device power, liquid nicotine concentration and nicotine form on nicotine delivery, and the present study.

ECIGs: an overview

Electronic cigarettes heat a liquid that often contains nicotine to create an inhalable aerosol. Patented in 2003 (Hon, 2003), ECIGs have increased in popularity in recent years (King et al., 2018) and have evolved considerably from their original design (Voos et al., 2019). One way of characterizing this evolution is to describe ECIGs by "generations" of products (e.g., Breland et al., 2018), in which the original generation are classified as disposable "cig-a-likes" (because they look like a combustible cigarette); the second generation includes a rechargeable battery and, often, a reusable cartridge/tank that holds the liquid; the third generation involves modular or "mod" components such as interchangeable heating elements and other features that allow the user to control device power, and the most recent generation involves ECIGs that use small, disposable liquid-filled pods (that also hold the heating element) and so are called "pod based" or "pod mod" ECIGs. Research involving each generation of ECIGs reveals the characteristics of the devices (e.g., electrical power; Wagener et al., 2017; Talih et al., 2019), the liquids used in them (Behar et al., 2017; Goniewicz et al., 2014; Omaiye et al., 2019), and the nicotine delivery profile of some device/liquid combinations (e.g., Dawkins & Corcoran, 2014; Harvanko et al., 2020a; Wagener et al., 2017; St. Helen et al., 2016; Hiler et al., 2020; Vargas et al., 2020). While first generation ECIGs delivered very little or no nicotine (Bullen et al., 2010; Vansickel et al., 2010), other more modern devices such as third generation "mods" and fourth generation "pod mods" have the potential to deliver as much or more nicotine than a combustible cigarette (Hiler et al., 2020; Yingst et al., 2019).

In addition to studying device characteristics and nicotine delivery profile, several clinical trials have examined ECIGs as a smoking cessation aid, revealing, at least in some cases, a modest improvement in cessation rates relative to NRT (Hajek et al., 2019; Walker et al.,

2020). While these clinical trial results suggest some efficacy, increasing use of ECIGs by young people (Gentzke et al., 2019), particularly those who never smoked combustible cigarettes (Soneji et al., 2017), is alarming and a potential threat to public health (Maziak, 2020). Also alarming is that, as ECIG products have evolved, ECIG devices have increased in their electrical power output (Farsalinos et al., 2014; Wagener et al., 2017) that may lead to greater user toxicant exposure (El-Hellani et al., 2018) and ECIG liquids have increased in their nicotine concentration (Walley et al., 2019). This increase in liquid nicotine concentration has been accompanied by a shift from the more aversive free-base form of nicotine to the less aversive protonated form (Jackler & Ramamurthi, 2019). Together, increased nicotine concentration and decreased aversiveness may increase the likelihood of ECIG-induced nicotine dependence (Huang et al., 2019). Unfortunately, little is known about how increased device power, greater liquid nicotine concentration, and nicotine form (i.e., freebase versus protonated) influence the nicotine delivery profile of the aerosol produced from an ECIG. Each of the topics discussed briefly above are detailed in the following sections.

What are ECIGs?

In general, an ECIG consists of four components: a source of electrical power (usually a battery), a reservoir to hold a liquid (e.g., “cartridge”, “tank”, or “pod”), and a heating element or “coil” that is sometimes a separate component and other times is an integrated part of the reservoir. The liquid that is placed in the reservoir often contains nicotine along with propylene glycol, vegetable glycerin, and flavoring agents. When the battery is activated, either by puffing

on the device or pressing a button, electrical power flows through the coil to heat the liquid and the inhalable aerosol is produced.

First generation or "cig-a-likes" are designed to look like a cigarette and often are disposable and cannot be refilled with liquid. The heater generally is activated when the user puffs on the mouth end. At least as originally marketed, cig-a-likes often failed to deliver nicotine effectively. For example, in a study of 32 ECIG-naive combustible cigarette smokers, participants took ten puffs (30 second inter-puff interval, or IPI) during each of four study conditions: an own brand cigarette, a cig-a-like ECIG with a 16 mg/ml liquid, a different cig-a-like ECIG with an 18 mg/ml liquid, and an unlit combustible cigarette (Vansickel et al., 2010). The lit combustible cigarette condition was a positive control that served to show typical nicotine delivery of a combustible cigarette under normal conditions. The unlit combustible cigarette was a negative control that served to control for the act of inhaling the ten puffs without any nicotine delivery. When participants used their own brand of cigarette, mean plasma nicotine concentration increased significantly from 2.1 ng/ml (SD=0.3) at baseline to 18.8 ng/ml (SD=11.8) immediately after the 10^{th} puff. This nicotine delivery profile of a combustible cigarette is similar to that observed in other studies after similar puffing conditions (e.g., 15-20 ng/ml; Breland et al., 2002; Cobb et al., 2010). Mean plasma nicotine peak change from baseline was not significant in either the 16 mg/ml condition (0.5 ng/ml) or the 18 mg/ml condition (1.4 ng/ml). No significant differences were observed when comparing mean plasma nicotine concentration after 10 puffs in either of the ECIG conditions to 10 puffs from the unlit tobacco cigarette. In another study of 23 experienced ECIG users, participants took 10 puffs (similar to Vansickel et al., 2010, described above) during two study conditions: a first generation ECIG and a second generation ECIG (Farsalinos et al., 2014). Both conditions in this study used the

same 18 mg/ml ECIG liquid. When participants used the first generation ECIG, mean plasma nicotine increased significantly from 2.8 ng/ml (SD=0.4) at baseline to 4.9 ng/ml (SD=0.5) immediately following 10 puffs. When participants used the second generation ECIG, mean plasma nicotine increased significantly from 2.5 mg/ml (SD=0.3) at baseline to 6.6 ng/ml (SD=0.6) following 10 puffs. Mean plasma nicotine concentration was significantly greater when participants used the second generation ECIG but were still lower than those obtained after smoking a tobacco cigarette under similar conditions (e.g., Vansickel et al., 2010). Other clinical laboratory studies of first generation ECIGs reported no significant nicotine delivery (Bullen et al., 2010) or very little nicotine delivery (Dawkins & Corcoran, 2014; Nides et al., 2014). Overall, compared to combustible cigarettes, cig-a-likes generally are not effective at nicotine delivery.

Second generation ECIGs are typically larger than cig-a-likes and contain a refillable reservoir (often called a cartridge or tank) that also contains the heating element which, in some cases, can be replaced (Breland et al., 2018). Device power (a function of battery voltage and heating element resistance) of second generation ECIGs is sometimes higher than that of cig-a-likes. Typically, the nicotine used in these devices is available in similar concentrations (0 mg/ml to ≈20 mg/ml; though sometimes slightly greater, up to ≈36 mg/ml) and form (freebase) as first generation devices. Studies of cigarette smoke suggest that freebase nicotine (pH ≈9) is more easily absorbed when compared to protonated nicotine (pH ≈6, El-Hellani et al., 2015; Pankow, 2001).

Second generation ECIGs sometimes deliver more nicotine when compared to first generation devices (e.g., Farsalinos et al., 2014, described above). In a clinical laboratory study using methods similar to Vansickel et al., 2010, 33 ECIG-experienced individuals completed

four ECIG ("eGo" 3.3-V battery with a 1.5 Ohm coil, device power of 7.3 W) use conditions (10 puffs; 30 second IPI) that differed by the nicotine concentration of the liquid nicotine that was placed into the ECIG cartridge: 0, 8, 18, or 36 mg/ml (Hiler et al., 2017). Plasma nicotine "boost" (the difference that results when baseline nicotine concentration is subtracted from post-puffing nicotine concentration) differed significantly across the 0, 8, 18, and 36 mg/ml conditions. In the 8 mg/ml condition, mean nicotine boost was 8.2 ng/ml (SD=7.8). As liquid nicotine concentration increased, nicotine boost also increased; mean boost in the 18 mg/ml condition was 13.0 ng/ml (SD=6.2) and in the 36 mg/ml condition was 17.9 ng/ml (SD=17.2). When a similar liquid nicotine concentration was used in first generation devices (18 mg/ml; see Vansickel et al., 2010 detailed above), nicotine delivery was not significantly greater than that of an unlit cigarette. In this study using second generation devices, nicotine boost was significantly greater in the 8, 18, and 36 mg/ml conditions when compared to the 0 mg/ml condition. Second generation ECIGs can sometimes deliver nicotine to the user, and the amount of nicotine delivered depends, at least in part, on the concentration of nicotine in the liquid that is placed into the ECIG reservoir. Similar results have been observed in other studies involving second generation devices (e.g., Wagener et al., 2017). The increased power and nicotine concentration when compared to first generation devices may result in the observed increase in nicotine delivery. Overall, reliable nicotine delivery can be observed in these second generation devices, approaching the nicotine delivery of a combustible cigarette in some conditions (e.g., 36 mg/ml nicotine in a ≈7 W device; 10 puffs with a 30 sec IPI).

Third generation ECIGs often have a larger battery (for longer use between charges) and a larger tank (that holds more liquid) compared to previous generations (Breland et al., 2018). These devices are also referred to as "mods," or "box mods" due to the ability of the user to

customize the power of the device as well as the concentration and form of liquid used (Breland et al., 2018). In a study using similar methods described previously (Hiler et al., 2017; Vansickel et al., 2010), the nicotine delivery profile of second and third generation ECIGs were compared (Wagener et al., 2017). ECIG experienced users who reported using either a second or third generation ECIG for ≥ three months were recruited and used their preferred device for this study. Nine second generation and 11 third generation users completed the study, and their personal ECIG devices were used for an initial 5 minute, 10-puff bout (30 second IPI) followed by 115 minutes of *ad libitum* use. After 10 puffs, mean plasma nicotine concentration was significantly less in the second generation group, at 7.3 ng/ml (SD=2.8), relative to the third generation group, at 17.5 (SD=12.9). These differences are likely due to differences in liquid nicotine concentration and device power. Indeed, second generation devices used in this study had mean liquid nicotine concentration of 22.3 mg/ml (SD=7.5) and device power of 8.9 W (SD=1.9). Third generation devices had a significantly lower mean liquid nicotine concentration of 4.1 mg/ml (SD=2.9), and higher mean device power of 71.6 W (SD=50.0). Mean device power was approximately eight times higher in the third generation group compared to the second generation group. This difference in device power may be responsible for the increased nicotine delivery observed in third generation devices, despite the observation that the third generation devices were filled with a liquid that had a nicotine concentration that was significantly lower than the second generation devices. Consistent with this suggestion, research from an aerosol research laboratory study using a machine-puffing protocol reveals that, when all other factors are held constant, increasing power by a factor of 2.5 (i.e., from 3 to 7.5 W) leads to a three- to four-fold increase in nicotine emissions (Talih et al., 2015).

In a study in which power was manipulated systematically (Hiler et al., 2020), plasma nicotine again increased as device power increased. ECIG experienced users (N=32) used an ECIG (4.5-V Kanger SUBOX; ECIG use similar to previously described studies, i.e., Vansickel et al., 2010) for each of four study conditions that differed by device power and liquid nicotine concentration (40.5 or 13.5 W; 3 or 8 mg/ml). In the 40.5 W+8 mg/ml condition, mean plasma nicotine concentration increased significantly from 2.7 ng/ml (SD=2.6) at baseline to 10.2 ng/ml (SD=8.2) following 10 puffs. In the 40.5 W+3 mg/ml condition, mean plasma nicotine increased significantly from 2.5 ng/ml (SD=1.5) at baseline to 7.0 ng/ml (SD=5.0). In the 13.5 W+8 mg/ml, mean plasma nicotine increased significantly from 2.5 ng/ml (SD=1.9) at baseline to 7.1 ng/ml (SD=8.7), and in the 13.5 W+3 mg/ml, mean plasma nicotine concentration did not differ significantly from baseline after 10 puffs. Mean plasma nicotine concentration in the 40.5 W+8 mg/ml condition was significantly higher than all other conditions after ten puffs. The systematic manipulation of power and liquid nicotine concentration in this study shows that both power and liquid nicotine concentration contribute to the nicotine delivery profile of ECIGs. Specifically, as device power is increased, the nicotine delivery profile is increased; the same is true for liquid nicotine concentration. Similar studies are consistent with these findings (e.g., Harvanko, et al., 2020a). Overall, as with second generation devices, “mods” are capable of reliable nicotine delivery, especially when device power is high; in such cases, their nicotine delivery profile approaches that of a combustible cigarette (15-20 ng/ml; see Vansickel et al., 2010 above).

The most popular ECIG used in the United States is JUUL, a “pod mod” style device (King et al., 2018; Vallone et al., 2019). These devices use disposable “pods” that combine the heating element and liquid. Though JUUL is the most popular brand of pod mod, there are a number of similar products commercially available, including disposable devices that do not fall

under current federal flavor regulations (FDA 2019; Williams, 2019). Pod mod devices typically have less power than third generation ECIGs (e.g., ≈8 W; Talih et al., 2019). The liquid nicotine concentration is often high (50-60 mg/ml), and typically consists of protonated nicotine (Talih et al., 2019) as opposed to liquids used in earlier generation devices which consists of freebase nicotine. Freebase nicotine, as described above, can be aversive at high concentrations because it is absorbed preferentially in the upper respiratory tract, leading to irritation (Henningfield et al., 2004; Pankow, 2001). Protonated nicotine is a combination of the freebase that is chemically bound with a proton from an acid (i.e., benzoic acid) which forms a salt. Nicotine salt is carried with the aerosol generated by the ECIG past the upper respiratory tract and is deposited deep in the respiratory tract. Therefore, the irritation from the freebase is largely attenuated, making protonated nicotine less aversive at high concentrations (Pankow, 2001). Although freebase nicotine is more easily absorbed (based on studies of tobacco cigarette smoke), protonated nicotine allows the user to inhale a larger volume of high concentration nicotine aerosol, which may lead to increased nicotine delivery (Brunnemann & Hoffmann, 1974; St. Helen et al., 2017).

In a study of experienced JUUL users, participants completed two 60-minute *ad libitum* ECIG use periods (preferred flavor and tobacco flavor; Vargas et al., 2020). In the preferred flavor condition, mean plasma nicotine increased significantly from 1.8 ng/ml (SD=0.4) at baseline to 10.9 mg/ml (SD=1.5) following ECIG use. In the non-preferred flavor condition, mean plasma nicotine increased from 2.02 ng/ml (SD=0.4) at baseline to 10.4 ng/ml (SD=1.6) following ECIG use. In another study comparing JUUL and IQOS (a heated tobacco product that is not considered an ECIG) in 18 cigarette smokers, participants took 10 puffs (30 second IPI) from a product in each study condition: JUUL, IQOS, or own brand (OB) combustible cigarette (Maloney et al., 2020). When participants used OB, mean plasma nicotine concentration

significantly increased from 2.1 ng/ml (SD=0.2) at baseline to 20.4 ng/ml (SD=20.4) following 10 puffs. When participants used JUUL, mean plasma significantly increased from 2.2 ng/ml (SD=0.7) at baseline to 9.8 ng/ml (SD=4.9) following 10 puffs. When participants used IQOS, mean plasma nicotine significantly increased from 2.1 ng/ml (SD=0.2) at baseline to 12.7 ng/mL (SD=6.2) following 10 puffs. Mean plasma nicotine concentration was significantly greater in the OB condition, compared to the JUUL and IQOS conditions.

An additional study of dual users (ECIG users who also smoke combustible cigarettes) examined the nicotine delivery profile of JUUL and combustible cigarettes following 5 minutes of *ad libitum* use (Hajek et al., 2020). Maximum plasma nicotine concentration was achieved during use (at 4 minutes) for both conditions. Mean maximum plasma nicotine concentration (C_{max}) for JUUL was 20.4 ng/ml (SD=15.0), and combustible cigarette C_{max} was 19.2 ng/ml (SD=17.6). This comparable nicotine delivery profile of JUUL relative to the combustible cigarette condition may be attributed to familiarity with the device; in previous studies, the nicotine delivery profile of JUUL was assessed in combustible cigarette smokers (e.g., Maloney et al., 2020). In this study, participants had experience using ECIGs, and regularly used both ECIGs and combustible cigarettes. ECIG-experienced individuals take longer puffs from ECIGs than ECIG-naïve combustible cigarette users (Hiler et al., 2017) and longer puff duration leads to more nicotine being emitted from the mouth-end of an ECIG (Talih et al., 2015). Pod mod devices reliably deliver nicotine, though the nicotine delivery profile can be less than that of a combustible cigarette after 10 puffs in ECIG-naïve individuals. However, the nicotine delivery profile of pod mod devices may reach that of a combustible cigarette in users that are familiar with the device, likely because these users take longer puffs. Similar studies have observed a similar nicotine delivery profile in pod mod devices (e.g., Yingst et al., 2019; Wynne et al.,

2018). Pod mod ECIGS are lower powered devices compared to third generation ECIGs, yet they are able to deliver nicotine in similar ways. Their high nicotine delivery profile likely is due to the high liquid nicotine concentration that users find less aversive to inhale because the nicotine is in the protonated from. This combination of nicotine delivery and palatability, along with marketing and product design, likely contributed to the rapid growth in popularity of pod mod ECIGs (Huang et al., 2019; Ickes et al., 2020; Vargas et al., 2020).

Additionally, ECIG nicotine delivery profiles have been compared within generations, revealing considerable heterogeneity. In a study comparing nicotine delivery across fourteen different devices (including first, second, and third generation) characterized as "first generation" and "advanced generation," differences were observed within the "advanced" category (Yingst et al., 2019). In this study, 14 ECIG users used their own devices (either first generation or advanced generation with liquid nicotine concentration >12 mg/ml) for 30 puffs (20 second IPI). Mean nicotine boost for first generation devices was 1.8 ng/ml (SD=0.9), compared to 10.8 ng/ml (SD=9.8) for advanced generation ECIGs. While some variability was observed for first generation devices, visual inspection of the plasma nicotine figure (Figure 2 in Yingst et al., 2019) reveals eight of the ten advanced generation ECIGs with nicotine boost less than 10 ng/ml and two advanced devices resulting in nicotine boost over 20 ng/ml. Additionally, within-generation variability was observed in a study described previously (Wagener et al., 2017). Significant differences were observed between second and third generation devices, however significant variability in mean plasma nicotine concentration was observed in the third generation group, with a large standard deviation (12.9) around a mean of 17.5 that suggested heterogeneity in nicotine delivery within this group of products. In both of these studies, significant differences are observed between ECIG generations, but importantly, significant

variability observed within generation may make nicotine delivery profile characterization by generation an oversimplification.

While early ECIG devices were sometimes shown to deliver very little nicotine (Bullen et al., 2010; Vansickel et al., 2010), other contemporary devices can deliver as much or more nicotine than a combustible cigarette (Hajek et al., 2020; Hiler et al., 2017; Wagener et al., 2017). These changes are important to monitor and understand thoroughly, and extant data make clear that characterizations based solely on generation do not predict the nicotine delivery of a device. Instead, understanding the influence of device characteristics and user behavior on nicotine delivery can lead to a more robust understanding of these products, who uses them and why, and the impact they may have on public health.

Who uses ECIGS and why?

Globally, the value of the ECIG market is $12.3 billion (only 1-2% of the global value of the combustible cigarette market; Kennedy et al., 2017). The countries with the largest share of the ECIG market are the United States, Russia, and Germany (Kennedy et al., 2017). In the United States, 3.2% of adults and 20.8% of youth (under 18) reported past 30-day ECIG use in 2018 (Bao et al., 2020; Cullen et al., 2018). A dramatic increase in ECIG use has been observed among youth in the United States, with an increase from 1.5% in 2011 to 20.8% in 2018 (Cullen et al., 2018). Rates of ECIG use among adults have remained relatively stable, ranging from 3.7% in 2014 to 3.2% in 2018 (Bao et al., 2020). As previously noted, ECIGs have been described as a public health advantage capable of increasing combustible cigarette smoking

cessation rates (Hajek et al., 2019; Walker et al., 2020) as well as a threat to public health due to increasing use by youth (FDA, 2018; Maziak, 2020).

ECIGs have been considered as a cessation aid or reduced harm product because of their potential ability to deliver nicotine with reduced exposure to toxicants when compared to combustible cigarettes (Goniewicz et al., 2017; Hartmann-Boyce et al., 2018b; St. Helen et al., 2020). In a study using 2014 and 2015 data from the cross-sectional National Health Interview Survey (NHIS) examining current and recently former smokers (smokers who quit during or after 2010, N=15,532), a quarter of survey respondents (N=3739) had quit smoking at the time of the survey (Giovenco & Delnevo, 2018). Among these former smokers, 10.3% were daily ECIG users, compared to 3.3% daily ECIG users in the group of current smokers. Daily ECIG use was reported in 5.1% of the entire sample, and within this group over half (52.5%; N ≈416) reported that they quit smoking cigarettes in the last 5 years. Daily ECIG use was the strongest predictor of prolonged combustible cigarette smoking abstinence in this study that involved self-reported tobacco use behavior and no objective verification of smoking status. This study suggests that daily ECIG use may increase the likelihood of successful cessation of combustible tobacco use.

In a study using data from Waves 1-3 (2013-2016) of the Population Assessment of Tobacco and Health (PATH) longitudinal cohort study, reported ECIG use at Wave 1 was examined as a possible predictor of reported abstinence from smoking combustible cigarettes at Wave 2 and 3 (Kalkhoran et al., 2020). Among respondents who reported daily combustible cigarette use at Wave 1, 22% were also daily ECIG users. Daily ECIG use at Wave 1 was significantly associated with prolonged abstinence from combustible cigarette smoking at Waves 2 and 3. There was no significant association between non-daily ECIG use and prolonged combustible cigarette smoking abstinence. Though these two studies give insight into the

potential relationship between daily ECIG use and cessation of combustible cigarette smoking, they do not provide evidence on the clinical efficacy of ECIGs as a combustible smoking cessation aid. In a meta-analysis examining ECIG use and combustible cigarette smoking cessation among adult combustible cigarette smokers, 20 studies with control groups were examined (Kalkhoran & Glantz, 2016). The studies examined included 15 cohort studies, three cross-sectional studies, and two clinical trials (both detailed below). Overall, ECIG use was associated with a 28% decrease in odds of quitting cigarettes compared with those who did not use ECIGs. Importantly, this decrease in odds is not consistent with individual results from a number of studies. This inconsistency suggests that until data are examined together, the observed benefit of ECIGs in combustible smoking cessation in individual studies may be an overestimation.

Though controlled, prospective research on ECIG use as a combustible smoking cessation aid is sparse, there are three relatively large-scale, double-blind, randomized controlled trials (RCTs) that have examined the efficacy of ECIGs when used as a combustible smoking cessation aid. In a 2013 RCT, 657 participants were randomly assigned to three groups, and were given either: 21 mg nicotine patches (N=295, one per day; NRT), a 16 mg/ml ECIG (N=289), or a placebo ECIG (N=73; containing no nicotine). Abstinence was verified via exhaled breath carbon monoxide (CO) at six months post-quit day (Bullen et al., 2013). Behavioral support was also offered via telephone and/or SMS from a national quitline. The ECIG used in this study was a first generation ECIG, and four participants completed a testing procedure after one week of ECIG use with the active device that contained the 16 mg/ml nicotine liquid. During this testing procedure, plasma nicotine concentration increased from 2.1 ng/ml at baseline to a peak of 3.4 ng/ml after ten minutes of product use. The nicotine delivery observed in this testing period was

similar to the nicotine delivery profile observed in other first generation devices (see Farsalinos et al., 2014, detailed above), and an order of magnitude less than typically seen after 10 puffs from a combustible cigarette (e.g., Vansickel et al., 2010). Overall, 78% (N=513) of participants completed follow-up at six months. Abstinence at six months after quit day (verified by CO) was 7.3% in the 16 mg/ml ECIG group, 5.8% in the NRT group, and 4.1% in the placebo ECIG group. No between-group differences were statistically significant. Thus, this study does not provide evidence that ECIGs (with or without nicotine) are more or less effective than NRT for combustible cigarette smoking cessation.

In an RCT published in 2019, 1124 participants were assigned randomly to one of three groups: 21 mg nicotine patches (N=125, one per day; NRT), NRT plus an 18 mg/ml ECIG (N=500), or NRT plus a placebo ECIG (N=499, containing no nicotine). Abstinence was verified (via eCO) six months after quit day (Walker et al., 2020). Weekly behavioral support was provided via phone for the first six weeks. The ECIG used in this study was a second generation ECIG, paired with either 0 mg/ml or 18 mg/ml liquid nicotine concentration. Participants were permitted to seek out new ECIGs or liquids if desired. During the trial, 15% of the NRT only group had used an ECIG during the trial, and 11% of the NRT plus 0 mg/ml ECIG group had switched to using an ECIG containing nicotine. Overall, 69% of participants completed follow-up at six months following their quit date. Among those participants, six-month abstinence rates were 7% in the NRT plus 18 mg/ml ECIG group, 4% in the NRT plus 0 mg/ml ECIG group, and 2% in the NRT only group. Abstinence rates in the NRT plus 18 mg/ml ECIG group were significantly higher than both the NRT plus 0 mg/ml ECIG group and the NRT only group. These results suggest a small but reliable improvement in combustible cigarette cessation when using a nicotine-containing ECIG in combination with NRT and six weeks of telephone

counseling. Though these results suggest that using a second generation ECIG device paired with NRT could increase the likelihood of successful combustible cigarette abstinence, the lack of testing of the nicotine delivery profile of the device used and the high loss to follow-up rate (31%) should be noted when considering the results observed in this study. Additionally, this study involved the use of telephone counseling and NRT, neither of which are available at retail venues where ECIGs are sold (i.e., "vape" shops), where most ECIG users purchase ECIG products (Braak et al., 2019).

In an RCT published in 2020, 886 participants were randomly assigned to two groups (ECIG, N=439, or NRT, N=447) and provided with either a second generation ECIG or their preferred NRT product on their quit date (Hajek et al., 2019). The ECIG used in this study was a "OneKit," a beginner ECIG kit with instructions on how to use/refill the device. Specifically, this device consisted of a 2.1-ohm atomizer and 650-mAh battery and included one bottle of 18 mg/ml nicotine liquid. 42 participants used a different ECIG that consisted of a 1.5-ohm atomizer and 1000-mAh battery due to the discontinuation of the original "OneKit" during the trial. Weekly one-on-one behavioral support provided by a clinician was offered for at least four weeks. Overall, 78.8% of participants completed follow-up at one year after their quit date. Among those participants, the 1-year abstinence rate in the ECIG group was 18.0%, compared to 9.9% in the NRT group. These results suggest ECIGs could be a more effective combustible cigarette cessation aid than NRT, at least in the context of individualize counseling. These results also suggest that using a second generation ECIG device could increase the likelihood of prolonged successful combustible cigarette abstinence, though importantly, the nicotine delivery profile of this ECIG was not tested. The loss to follow-up was comparable to other studies (i.e.,

Bullen 2013 above), and this loss is important to consider when evaluating the efficacy of ECIGs as a combustible cigarette smoking cessation aid.

Considering these three RCTs together, there is growing evidence for the potential efficacy of at least some ECIGs when used as a combustible smoking cessation aid when they are paired with cessation counseling (either via phone or individualized and in person) and, perhaps, with NRT. Importantly, even the study with the greatest effect (Hajek et al., 2019) reports a treatment failure rated of >80%. Clearly, additional RCTs are needed to establish under what conditions and for whom ECIGs are effective as cessation aids. Such studies would be most informative if the nicotine delivery profiles of the ECIG device/liquid(s) used were established prior to participant enrollment and the ability of the device/liquid(s) to deliver nicotine effectively was part of the rationale for inclusion of these products in the RCT. While there is some evidence that ECIGs can help treatment-seeking, cigarette-smoking adults to quit smoking, adult combustible cigarette smokers are, unfortunately, not the only group using ECIGs.

As previously described, ECIG use among youth has increased dramatically, from 1.5% in 2011 to 20.8% in 2018 (Cullen et al., 2019). During this same time period, combustible cigarette use among youth has declined from 15.8% to 8.1% (Gentzke et al., 2019). This increased prevalence in ECIG use is correlated directly with the marketing of pod mod devices that contain protonated nicotine, including JUUL (a low-wattage device with a ≈60 mg/ml nicotine concentration liquid containing 94% protonated nicotine; Talih et al., 2019). Pod mod devices are especially popular with youth, likely due to a combination of marketing, design that facilitates concealed use, and lack of aversive properties upon inhalation due to the use of protonated nicotine (Glasser et al., 2021; Ickes et al., 2020; Pankow, 2001). As detailed in Figure

1, as ECIG use has increased among youth, a public health threat emerges, particularly when nicotine-naïve youth (or youth that otherwise would not have used tobacco products) begin to use ECIGs. Indeed, evidence is emerging of nicotine-naïve youth using ECIG devices containing protonated nicotine (Krishnan-Sarin et al., 2019). While ECIGs may provide a public health benefit when used as combustible cigarette smoking cessation aids, increased popularity among youth has the potential to negate any public health benefit by initiating nicotine dependence in young people.

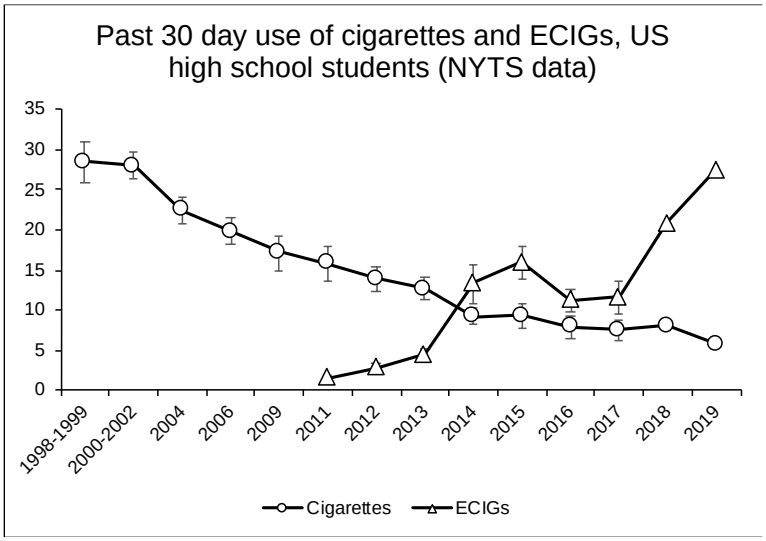

Figure 1. Past 30-day use of cigarettes and ECIGS in US high school students, 1998-2019. Data taken from the National Youth Tobacco Survey (CDC).

In a cross-sectional survey study, 371 undergraduate university students reported reasons for use of a protonated nicotine containing ECIG device (Ickes et al., 2020). Overall, 36% of participants reported ever use of protonated, nicotine-containing ECIGs. Current (past 30-day) use of these devices was reported by 21% of the sample. The two most popular reasons for use among ever users were curiosity (95%), and "my friends use it" (81%). These reasons indicate an awareness of the product and popularity with peers as primary motivators to initiate use of these products. The most popular reasons for use among current users were: "ease of use" (91%), "doesn't smell bad" (87%), portability (85%), stress/relaxation (82%). These reasons suggest

that maintained use of these products may be motivated by device characteristics and nicotine dependence. While youth and young adults may initiate use of protonated nicotine containing ECIGs due to the desire to experiment with a product they are aware of (likely through marketing) and to fit in with their friends, the maintenance of use may depend on specific device characteristics (ease of use, portability) and nicotine dependence (relief of stress/abstinence symptoms). Considering these reasons for use makes clear that ECIGs containing protonated nicotine may have created a user base of young people who might otherwise have avoided combustible tobacco products and thus exposure to nicotine.

In a study of students from 4 Connecticut high schools in 2017, 875 students responded that they had used at least one of the four ECIG generation devices (Krishnan-Sarin et al., 2019). Mod devices were the most popular (71.2%) among ever users. Among current users, a pod mod device containing protonated nicotine was the most popular (47.1%). Ever use of the protonated nicotine containing pod mod device was not associated with other tobacco product use (e.g., cigarettes, cigars, cigarillos, hookah, or smokeless tobacco), suggesting that users of this type of device may have been nicotine-naïve upon initiation of use. Current use of protonated pod mod devices was associated with a higher socio-economic status (SES) when compared to those who did not use this type of device. Interestingly, SES could also contribute to likelihood of other tobacco product use, as lower SES previously has been established as a predictor of tobacco use (Gilman et al., 2003; Mathur et al., 2013; Wang et al., 2018). In order to understand how initial use of ECIG devices containing protonated nicotine may contribute to later use of other tobacco products, the reasons for initial use in youth and young adult users must be explored. Additionally, the relationship between ECIG use and subsequent use of other tobacco products must be examined.

In a longitudinal study of college-aged students, the relationships between ECIG use and subsequent combustible cigarette use was examined (Spindle et al., 2017). Among those who reported ever trying e-cigarettes at baseline (N=153), approximately a quarter (24.2%; N=37) reported having tried cigarettes one year later. Ever ECIG users at baseline were over three times more likely to report trying cigarettes one year later. This study demonstrates that ECIG use could lead to an increase in the likelihood of combustible cigarette use. Similar results were observed in a review of four longitudinal studies, where participants who reported ECIG use at baseline were 3-5 times more likely to use combustible cigarettes at follow-up (12-15 months) when compared to non-ECIG users (Chatterjee et al., 2018). These studies indicate that ECIG use increases the likelihood of combustible cigarette smoking, an especially alarming result considering the high rate of ECIG use in youth and young adults (detailed above). When considering reasons for initiation and continued use of ECIGs, and the increased vulnerability to use other tobacco products, youth ECIG use becomes an important public health consideration.

Evidence from individual studies suggests that ECIGs may benefit public health when used to aid cessation of combustible cigarette smoking, though when data are examined together, the use of ECIGs as a combustible cigarette cessation aid is associated with a decreased likelihood of success (Kalkhoran & Glantz, 2016). The public health threat of ECIG use among youth and young adults must also be considered. Especially imperative is the threat of ECIG use in otherwise nicotine-naïve youth, and how that use may lead to nicotine dependence and later use of combustible cigarettes. Some research has suggested that the potential life-years gained from ECIG use may surpass the life-years lost from youth use and subsequent vulnerability to combustible tobacco use (Warner & Mendez, 2019), while others have suggested that the popularity of ECIGs among youth could negate any progress made on reducing combustible

smoking rates (NASEM, 2018). Considering these two competing ideas regarding the influence of ECIG use on public health, any regulation of ECIGs must keep in mind the different types of users and their reasons for use. In order to regulate ECIGs comprehensively, a thorough understanding of device characteristics and how they influence nicotine delivery is imperative.

Protonated nicotine

Nicotine exists in unprotonated (freebase) form or ionized, protonated form. Protonation can be achieved by altering the pH of nicotine with the addition of an acid (e.g., benzoic acid), creating a salt. The dissociation constant (pKa) of a molecule allows a prediction to be made regarding dissociation/ionization based on pH (Hill & Petrucci, 2002). Specifically, dissociation (i.e., deprotonation) can be predicted based on a pH higher than the pKa, a dissociation constant (50% deprotonated, 50% protonated) can be predicted based on a pH equal to the pKa, and ionization (i.e., protonation) can be predicted based on a pH lower than the pKa of a certain molecule. The pKa of nicotine is 8.02 (Tomar & Henningfield, 1997). Therefore, freebase and protonated nicotine can be detected in ECIG liquid by measuring pH where a more alkaline pH >9 indicates mostly freebase nicotine, a more acidic pH <7 indicates mostly protonated nicotine (El-Hellani et al., 2015; Harvanko et al., 2020b), and a pH ≈8 will be approximately equal parts freebase and protonated nicotine (Tomar & Henningfield, 1997). Studies have established that pH modulates nicotine absorption; specifically, increased alkalinity increases nicotine bioavailability in smokeless tobacco products (Tomar & Henningfield, 1997). When considering combustible tobacco products, “flue-curing” in the 19th century effectively decreased tobacco pH compared to the standard “air-cured” tobacco, resulting in decreased harshness upon inhalation that in part led to the proliferation of combustible cigarettes (Slade, 1989; Milov 2019). In order to maintain bioavailability of nicotine in combustible cigarettes, blends of air- and flue-cured

tobacco were engineered to maximize nicotine exposure while simultaneously minimizing discomfort due to harshness. Tobacco companies also used tobacco plants genetically modified to have increased nicotine content, compensating for any decreases in nicotine bioavailability due to protonation, and special protonated nicotine "sprays," concealing any increases in nicotine content (Kessler, 1994). In fact, the tobacco industry added levulinic acid to nicotine to create a salt (protonated nicotine) that was then sprayed on "low-yield" combustible cigarettes, effectively concealing any harshness that may result from the added nicotine (Kessler, 1994).

Turning to ECIGs, early ECIG liquids contained mostly freebase nicotine, and the harshness of the aerosol produced by heating these freebase liquids tended to limit their nicotine content. That is, when ECIG liquids contained nicotine that was majority of the freebase form, liquid nicotine sold for immediate use in an ECIG (i.e., not for "do-it-yourself" flavor mixing at home) tended to be no more than 36 mg/ml nicotine. Indeed, when freebase nicotine was common in ECIG liquids, ECIG-experienced individuals used lower rather than higher concentration liquids (e.g., Wagener et al., 2017; Harvanko et al., 2018).

However, ECIG liquids evolved to incorporate protonated nicotine in higher concentrations, mirroring the evolution of combustible cigarettes; a product with a relatively low nicotine delivery profile has been engineered to deliver more nicotine without any additional harshness via protonation, albeit at a much swifter pace (Duell et al., 2020). As previously mentioned, the most popular ECIG in the US is JUUL, a ≈8 W, pod mod device that uses pods containing 94% protonated nicotine liquid at higher concentrations than typically used when the nicotine is freebase (>60 mg/ml; Talih et al., 2019). Other available pods and pod mod devices contain similar concentrations of protonated nicotine (Jackler & Ramamurthi, 2019), and this type of device accounts for 75% of the ECIG market in the US (Huang et al., 2019).

Additionally, high concentration protonated nicotine liquid is available in bulk (Jackler & Ramamurthi, 2019), allowing it to be used in other types of devices including third generation devices that allow the user to adjust device power.

As discussed previously, protonated nicotine is less aversive at higher concentrations when compared to freebase nicotine (Henningfield et al., 2004; Pankow, 2001). The increase in concentration of liquid nicotine available for ECIGs observed since 2015, when JUUL was introduced, almost certainly is a direct result of the introduction of devices containing protonated nicotine (Romberg et al., 2019). In a recent study examining the proliferation of high concentration protonated nicotine liquids, over 100 brands of protonated nicotine liquids >50 mg/ml were identified (Jackler & Ramamurthi, 2019).

The high concentration of protonated nicotine in pod mod devices results in reliable nicotine delivery even with a considerably lower device power than third generation ECIGs (Hajek et al., 2020; Maloney et al., 2020; Vargas et al., 2020). Currently, protonated liquid is available in pods (meant to be used with low powered pod mod devices) and in bulk. The availability of protonated nicotine liquids in bulk allows this type of liquid to be used in any device with a refillable reservoir including those with variable voltage (i.e., third generation devices), and the resulting potential for dramatic increases in nicotine delivery pose a danger to users. As discussed previously, nicotine emissions increase as much as four times when device power is increased by a factor of 2.5 (Talih et al., 2015) and studies have demonstrated that nicotine delivery is increased as device power is increased (Hiler et al., 2020; Wagener et al., 2017). If experienced users of pod mod devices are able to obtain nicotine delivery comparable to combustible cigarettes (Hajek et al., 2020; detailed above) with device power <10 W, and third generation device power can exceed 40 W, the use of high concentration protonated

nicotine in third generation devices presents the potential for nicotine delivery that far exceeds that of a combustible cigarette. This high level of nicotine delivery is especially disturbing when considering that the majority of ECIG users are youth and young adults (Cullen et al., 2018; detailed above).

Increased nicotine delivery resulting from the use of protonated nicotine in ECIG devices could result in increased efficiency when ECIGs are used as combustible cigarette cessation aids. Though there are few RCTs examining ECIGs in this role, the use of ECIG devices with increased nicotine delivery could be responsible at least in part for increases in cessation rates at follow-up (e.g., first versus second generation, as in Bullen et al., 2013 and Hajek et al., 2019). Existing evidence that the efficacy of NRT is nicotine dose-dependent (Lindson et al., 2019) suggests that, for any cessation aid used that involves nicotine delivery, greater nicotine delivery to blood likely will lead to better treatment outcome.

The ability for ECIGs to deliver nicotine also is a concern for public health, and regulators have begun addressing this concern. For example, in 2014, the European Union (EU) limited ECIG liquid nicotine concentration to $\leq$20 mg/ml in order to ensure that ECIG nicotine delivery is comparable and not greater than that produced by a combustible cigarette (Kennedy et al., 2017). Similar ECIG regulations on liquid nicotine concentration have been suggested in the US (H.R. 4624, 2019), yet little is known about the nicotine delivery of ECIG products containing protonated nicotine. Existing studies have examined the role of liquid nicotine concentration in nicotine delivery (e.g., Hiler et al., 2017, detailed above), but these studies used liquids containing nicotine in freebase form. As indicated previously, the vast majority of devices used in the US contain protonated nicotine (Huang et al., 2019), and any ECIG

regulations that involve liquid nicotine concentration should be informed by studies examining the role of nicotine form on nicotine delivery.

Additionally, any ECIG regulations focused on only liquid nicotine content do not address the role of device power in determining nicotine delivery (Eissenberg et al., 2020); in fact, increasing the device power has the potential to negate any decreases to liquid nicotine concentration, effectively preserving nicotine delivery (as in Hiler et al., 2020; Wagener et al., 2017). If liquid nicotine concentration is limited in the U.S. to a similar threshold as the EU regulation, device power has the potential to be used to circumvent the intention of the regulation and achieve nicotine delivery that exceeds that of a combustible cigarette (Eissenberg et al., 2020). Alternatively, factors that influence nicotine delivery can be considered together to determine the rate at which nicotine is emitted from an ECIG (i.e., nicotine flux; Shihadeh & Eissenberg, 2015). Nicotine flux subsequently may be combined with additional factors (e.g., puff duration) to limit effectively the maximum dose of nicotine ECIG users are able to administer. A thorough understanding of the nicotine delivery profile and subjective effects of different concentrations of protonated nicotine liquids at different power settings is integral to creating regulations that are comprehensive; that is, effective regulations that decrease the abuse liability of ECIGs in order to deter youth initiation without limiting their potential efficacy as combustible cigarette cessation aids.

Statement of the Problem

The majority of ECIGs currently used in the US contain protonated nicotine liquids at high concentrations (Huang et al., 2019). The recent increase in ECIG use among youth and young adults occurred with the introduction of ECIG products containing protonated nicotine (Romberg et al., 2019). Previous studies have established that the nicotine delivery profile of

ECIGs is influenced by the concentration of nicotine in the liquid and the power of the device (Harvanko et al., 2020a; Hiler et al., 2019; Wagener et al., 2017), but these studies have explored this relationship using freebase nicotine liquids at typical concentrations at the time of study. The introduction and subsequent rise in popularity of devices containing protonated nicotine in high concentrations has created an urgent need for further research on the nicotine delivery profile and subjective effects of protonated liquid at different power and liquid nicotine concentration combinations.

The Present Study

This study used clinical laboratory methods to examine nicotine delivery and subjective effects of ECIGs containing protonated nicotine liquids at three concentrations and two device power settings. Additionally, puff topography was measured to examine how user experience and behavior may interact to influence nicotine delivery.

Statement of Hypothesis

Previous research has established that as ECIG device power and/or liquid nicotine concentration increases, nicotine delivery also increases. The hypothesis for this study was that when protonated nicotine is used at different liquid concentrations and device power settings, nicotine delivery will approach (or exceed) that observed in previous studies on ECIGs and combustible cigarettes. In order to understand how device power and liquid concentration influence nicotine delivery, these parameters must be studied systematically. This study controlled device power and liquid nicotine concentration to examine the influence of protonated nicotine containing liquid on nicotine delivery of ECIGs. Additionally, the hypothesis in this study was that user experience (e.g., harshness, direct effects of nicotine) would result in changes to puff topography.

Method

Participant Selection

Participants were recruited via word-of-mouth and Institutional Review Board (IRB)-approved community and/or internet advertisements. All screening and experimental sessions took place at the VCU Center for the Study of Tobacco Products (CSTP). Individuals were considered eligible for the study if they were healthy, aged 18-55, reported using inhaled tobacco products and were willing to abstain from tobacco/nicotine as required. Specifically, ECIG users were required to report use of ECIGs ≥3 months and use of ≥1 ml of liquid per day (or approximately one cartridge or one pod per day) at a nicotine concentration of ≥3 mg/ml and no use of tobacco cigarettes in the past 30 days. Cigarette smokers were required to report use of ≥ 10 cigarettes per day and no use of an ECIG in the past 30 days. Cigarette smokers were also required to have an expired air CO concentration at screening of at least 15 ppm and a 'positive' cotinine cassette result to verify nicotine use. Urine cotinine was measured for all participants at screening, and a positive test was required to verify nicotine use.

Participants were excluded if they reported a current, diagnosed chronic illness or psychiatric condition, or psychotropic medication use. Additionally, participants were excluded if they reported alcohol use >25 days, cannabis use >15 days, or any other illicit drug use (cocaine, opioids, benzodiazepines, and methamphetamine) in the past 30 days. Biologically female participants were excluded if they reported currently breast-feeding or if they tested positive for pregnancy (by urinalysis) at screening. Any participant reporting any intention to quit tobacco/nicotine use in the next 30 days was excluded and referred to cessation treatment. Individuals who reported using any other tobacco products (other than what is permitted per the inclusion criteria) on a weekly or more frequent basis were excluded.

Informed Consent and Screening

All participants completed a two-part screening process. Interested participants were able to make initial contact via telephone or website (both provided on advertisements) and answered questions about their health and current tobacco product use. Based on their answers, eligible participants were invited to the CSTP to complete an in-person screening visit, where they provided informed consent to participate in the screening and the study. After consent procedures, participants completed additional screening questionnaires on demographics, health status, and tobacco product use. All participants provided a urine sample to test for cotinine and to test for pregnancy in biologically female participants.

Participant Safety

The methods and procedures used in this study involved minimal risk. Similar methods and procedures have been used numerous times at the CSTP over the course of 20 years. Abstinence from nicotine for twelve hours could result in mild discomfort, but this discomfort is not medically dangerous. Blood drawing procedures involve minimal risk of bruising and/or infection at the catheter site; these risks were minimized by trained nursing staff and sterile procedures. Potential risks and/or side effects of using ECIGs were routine for the target population (users of inhaled tobacco products).

All CSTP staff maintain training on good clinical practices, including the protection of participants' safety and rights. Heart rate (HR) and blood pressure (BP) were monitored during each session. Sessions were ended prematurely if a participant's HR was below 50 or above 120, or if a participant's systolic BP was below 90 or above 140, at any point during the session. Data were not identified by name or initials; only an alphanumeric code is used as identification. All data are stored in a locked cabinet available only to CSTP staff.

Materials

Participants used a third generation ECIG, Kanger Sub Box Mini, set to either 15 watts or 30 watts, which contained either 10 mg/ml, 15 mg/ml, or 30 mg/ml nicotine-containing liquid. All ECIG devices and liquids were purchased at a local vape shop (AVAIL, Richmond VA). Liquids were verified independently for liquid nicotine content (within 0.2 mg/ml of labeled concentration) by VCU's Bioanalytical Analysis Core Laboratories. All liquids used were 30% propylene glycol and 70% vegetable glycerin, had a pH of < 7 (this pH indicates a majority of protonated nicotine, as detailed above), and were available in four flavors (Tobacco, Menthol, Fruit, Dessert). Participants sampled all flavors during screening and selected their preference for the duration of the study. Among the 21 participants included in final analyses, flavor choices were as followed: Tobacco (N=5), Menthol (N=7), Berry (N=8), and Dessert (N=1).

Procedures

After completing informed consent and all screening procedures, participants completed six sessions at VCU's CSTP. Each session was approximately 4 hours long and differed by the combination of wattage and liquid nicotine concentration. Sessions were ordered by Latin-square and occurred no more than 2 days per week. All sessions were separated by at least 48 hours. Participants were instructed to abstain from tobacco and nicotine containing products for $\geq$12 hours prior to each session. In order to verify overnight abstinence from any combustible tobacco products, participants' expired air CO concentration was required to be $\leq$10 ppm upon arrival to the CSTP for each session. Because ECIGs are non-combustible, and CO therefore is not an indication of ECIG use/abstinence, baseline plasma nicotine concentration was inspected retrospectively following analysis to identify any non-compliance with overnight abstinence. Plasma was analyzed after participants complete the study; therefore, a 1-hour waiting period

was completed before ECIG administration. This waiting period ensured that any participants who did not comply with overnight abstinence were abstinent for at least one hour before the session began. After the waiting period, HR and BP monitoring began and an intravenous catheter was inserted into a forearm vein. A blood sample of 7 ml was drawn, and participants completed computerized questionnaires reporting any nicotine abstinence symptoms and other effects (see below). Thirty minutes after the initial HR and BP measurement, participants were instructed to take 10 puffs of the ECIG; each puff was separated by 30 seconds. A CSTP staff member instructed the participant when to take each puff and verified compliance. After the tenth puff, another 7 ml of blood was sampled, and participants completed the same questionnaires. Participants completed two additional questionnaires following ECIG use: a computerized questionnaire reporting direct effects of ECIG use and a questionnaire using paper and pen assessing the flavor, harshness, and throat hit of the ECIG. Twenty minutes after the first ECIG use period ended, another 7 ml blood sampled was drawn and participants completed the same computer questionnaires. After completing the questionnaires, participants began an *ad libitum* ECIG use period, where they were instructed to use the ECIG as much or as little as they liked for 90 minutes. Following the *ad libitum* use period, a final 7 ml blood sample was drawn, and participants completed the same questionnaires that were completed following the first ECIG use period. After all questionnaires were completed, the catheter was removed, and the participant was paid according to the number of sessions they completed thus far. Payment escalation according to session number was used to encourage study retention. The escalation schedule was: US $50 for completing the first session, US $75 for completing the second session, US $100 for completing the third and fourth sessions, US $150 for completing the fifth

session, and US $175 for completing the sixth session. Thus, the total amount participants earned for completing the entire study was US $660.

Outcome Measures

Physiological measures. All blood samples were centrifuged and stored at -70°C. Analysis of plasma nicotine concentration took place at VCU's Bioanalytical Analysis Core Laboratories, using a limit of quantitation (LOQ) of 2 ng/ml (as in Maloney et al., 2019). Participants' HR was measured via pulse oximeter (Criticare systems) and expired air CO was measured with a BreathCO monitor (Vitalograph, Lenexa, KS).

Puff topography. Mouthpiece-based puff topography equipment, developed and manufactured at the American University of Beirut (AUB), was used to measure IPI, flow rate, puff number, duration, and volume. This equipment is designed specifically to measure the puff topography of ECIG use and has been used in a number of studies on ECIGs (e.g., Hiler et al., 2020; Spindle et al., 2018). Specifically, the topography equipment used in this study is designed to accommodate the slower flow rate associated with ECIG use and has been shown to have no significant influence on other measures collected in this study (see Spindle et al., 2015). In order to correct for any measurement error or noise, the topography recording software automatically corrected for the following: any two puffs separated by $\leq$300 ms (combined into one puff) and any puffs with a duration $\leq$300 ms (puff deleted). Mouthpieces were manufactured to fit the device used in the present study. Prior to each session, the mouthpiece was calibrated using an automatic digital flow calibrator, also designed and manufactured at AUB. Puff topography was measured and recorded continuously during each ECIG use period.

Subjective questionnaires. Three questionnaires were administered via computer using the Visual Analog Scale (VAS) format; for each item, a word or phrase was centered on a

horizontal line with “not at all” on the left of the line and “extremely” on the right. Participants clicked on any point of the line with a mouse/cursor and response scores reflected the percentage of the total line length measured from the left anchor. These questionnaires were administered before and after the two ECIG use periods, for a total of four times each session. The General Labeled Magnitude Scale was administered via paper and pen after each ECIG use period, for a total of two times each session.

Direct Effects of Nicotine. The direct effects and side effects of nicotine were assessed by the direct effects of nicotine scale, which consists of 11 items: “Confused,” “Dizzy,” “Headache,” “Heart Pound,” “Lightheaded,” “Nauseous,” “Nervous,” “Salivation,” “Sweaty,” and “Weak” (Evans et al., 2006; see Appendix A).

Direct Effects of ECIG Use. Adapted from the “Direct Effects of Tobacco” scale (Breland et al., 2006) to measure the subjective effects of ECIG use, this scale consists of 10 items: “Was the e-cigarette satisfying?,” “Was the e-cigarette pleasant?,” “Did the e- cigarette taste good?,” “Did the e-cigarette make you dizzy?,” “ Did the e-cigarette calm you down?,” “Did the e-cigarette help you concentration?,” “Did the e-cigarette make you feel more awake?,” “Did the e-cigarette reduce your hunger for food?,” “Did the e-cigarette make you sick?,” and “Would you like another e-cigarette right now?” (see Hiler et al., 2020).

Hughes-Hatsukami Withdrawal Scale. Severity of nicotine withdrawal and severity of abstinence symptom(s) was assessed by the Hughes-Hatsukami withdrawal scale, which consists of 11 items: “Anxious,” “Craving and e-cigarette/nicotine,” “Depression,” “ Difficulty concentrating,” “Drowsy,” “Hunger,” “Impatient,” “Irritable,” “Restlessness,” “Desire for sweets,” and “Urge to use an ECIG” (Hughes & Hatsukami, 1986).

General Labeled Magnitude Scale. The General Labeled Magnitude Scale (gLMS) was used to measure specific sensations associated with ECIG product use. Participants were instructed to draw a horizontal line indicating their level of sensation, and then write the corresponding number in a box (see Appendix C). The following ECIG-specific sensations were measured via paper and pen: "Flavor," "Harshness," and "Throat Hit" (Green et al., 1993). This scale uses a scale of 0-100 and sensation level descriptions at the following numbers: 0 ("No Sensation at All", 1 ("Barely Detectable"), 6 ("Weak"), 16 ("Moderate"), 35 ("Strong"), 53 ("Very Strong"), and the highest possible rating of 100 ("Strongest Imaginable Sensation of Any Kind").

Participant characteristics.

A total of 84 participants provided informed consent, and 25 of these were determined ineligible for study participation at the screening visit for failure to meet study criteria (e.g., use of other tobacco products, besides those specified in study criteria, in the last 30 days). Thirty-two participants completed at least one session but withdrew or were discontinued before study completion for the following reasons: failure to follow up (n=11), lack of venous access (n=6), failure to adhere to study protocol (i.e., unable to remain abstinent for 12 hours prior to each study session as evidence by expired air CO concentration >10 PPM upon arrival; n=3), and elevated blood pressure (n=3). Two participants voluntarily withdrew from the study (one for lack of venous access and one for scheduling conflicts). Additionally, 7 of the 32 participants who did not complete the study were unable to attend study sessions due to the COVID-19 pandemic. Twenty-seven participants completed all study sessions. Among these 27, five participants in the ECIG user group were determined (via plasma nicotine concentration) to be noncompliant (e.g., did not abstain from nicotine and tobacco products for 12 hours prior to at

least one session) and one participant was unable to give a blood sample during one session. Thus, a total of 21 participants (13 male, 7 female, 1 other) are included in the final analyses.

No significant differences in age, race, education, or employment were observed between groups (see Table 1). Collapsed across group, mean age (SD) was 28.8 (8.9) years. Fourteen participants were Caucasian, 4 were African American, 1 was Asian American, and 2 reported their race as "other" ("Arab/Middle Eastern" and "Latino"). Thirteen participants reported being currently employed, 6 reported being unemployed, and 2 reported their employment as "student." Mean (SD) expired air CO at screening was 2.5 (0.7) in the ECIG user group and 26.3 (11.5) in the cigarette smoker group.

Among the ECIG user group (n=11), participants reported using a mean (SD) volume of 3.8 ml (3.8) of ECIG liquid daily (mean volume for one participant was not included due to incomplete information on their preferred device) for a mean (SD) of 15.5 months (12.3). For reference, a JUUL pod (the most popular ECIG device at the time of this study; Huang et al., 2019) contains 0.7 ml of nicotine-containing liquid (Talih et al., 2019). The ECIG user group scored a mean (SD) of 2.2 (0.9) on the E-cigarette Dependence Scale (EDS; Morean et al., 2019). Flavor choice within the ECIG user group was as followed: Tobacco (n=5), Menthol (n=3), Berry (n=3), Dessert (n=0).

Among the cigarette smoker group (n=10), participants reported smoking a mean (SD) of 19.2 (8.0) cigarettes per day for 11.7 (7.5) years. The cigarette smoker group scored a mean (SD) of 5.6 (1.7) on the Fagerström Test of Nicotine Dependence (Heatherton et al., 1991) and a mean (SD) of 2.1 (0.8) on the Patient Reported Outcomes Measurement Information System (PROMIS) Short Form v1.0 – Smoking: Nicotine Dependence for All Smokers 4a (Edelen et al.,

2014, 2016). Flavor choice within the cigarette smoker group was as followed: Tobacco (n=0), Menthol (n=4), Berry (n=5), Dessert (n=1).

Table 1. Participant demographic information by group and for the entire sample. No significant differences between groups were observed for any of the characteristics displayed here.

Participant Demographics	*Mean (SD) or N (%)*		
	ECIG Users n=11	Cigarette Smokers n=10	Entire Sample N=21
Age	24.6 (6.6)	33.3 (9.1)	28.8 (8.9)
Gender			
Male	7 (64%)	6 (60%)	13 (62%)
Female	4 (36%)	3 (30%)	7 (33%)
Other	0	1 (10%)	1 (5%)
Race			
Caucasian	8 (73%)	6 (60%)	14 (67%)
African American	1 (9%)	3 (30%)	4 (19%)
Asian American	1 (9%)	0	1 (5%)
Other	1 (9%)	1 (10%)	2 (9%)
Education (years)	13.5 (1.5)	12.9 (1.5)	13.3 (1.5)
Employment			
Part or full time employed	7 (64%)	6 (60%)	13 (62%)
Unemployed	3 (27%)	3 (30%)	6 (29%)
Student	1 (9%)	1 (10%)	2 (9%)
Tobacco product use			
ECIG users			
Volume (ECIG liquid/day in ml)	3.8 (3.8) [a]		
Duration ECIG use (months)	15.5 (12.3)		
Cigarette smokers			
Cigarettes/day		19.2 (8.0)	
Duration cigarette use (years)		11.7 (7.5)	
Nicotine dependence			
ECIG users			
EDS	2.2 (0.9)		
Cigarette smokers			
FTND		5.6 (1.7)	
PROMIS		2.1 (0.8)	

[a] n = 10

Note: EDS=E-cigarette Dependence Scale; FTND=Fagerström Test for Nicotine Dependence; PROMIS=Patient Reported Outcomes Measurement Information System Short Form v1.0 – Smoking: Nicotine Dependence for All Smokers 4a; ECIG=electronic cigarette.

Data Analysis Plan

A previous CSTP study that manipulated both liquid nicotine concentration and power (Hiler et al., 2020) was used to estimate effect size for the current study, in order to complete *a priori* power analysis and determine adequate sample size. Using data from Hiler et al., 2020, plasma nicotine partial n^2 values for liquid nicotine concentration and device power were examined separately and the smallest partial n^2 for a main effect of device power (0.32) and a main effect of liquid concentration (0.36) was used to determine effect size (0.69 and 0.75). The predicted effect sizes were entered into a G*Power repeated measures ANOVA model for an *a priori* power calculation (Faul et al., 2007). Results revealed that for plasma nicotine concentration analysis 10 participants were required to detect a main effect of device power and nine participants were required to detect a main effect of liquid nicotine concentration (with power ≥80% given a Type I error rate of 0.05).

The referenced study did not detect a significant device power by liquid concentration interaction; the partial n^2 (0.15) of a significant device power by time interaction was used to determine effect size (0.42) and the number of participants needed (17) to detect interaction effects. The absence of a significant resistance by liquid concentration interaction in the referenced study could be due to the range of liquid nicotine concentration used (3 mg – 8 mg). The present study uses a much wider range of liquid nicotine concentration (10 mg – 30 mg); for this reason, the observed effect size is likely to be larger than that observed in Hiler et al., 2020.

Plasma nicotine concentration values lower than the LOQ (2.0 ng/ml; see *Physiological measures*) were replaced with 2.0 ng/ml. This approach is more conservative than replacing each value below the LOQ with zero and has been used in other studies that measure plasma nicotine concentration (e.g., Vansickel et al., 2010; Hiler et al., 2020; Maloney et al., 2020). Heart rate

data were averaged for 10 minutes before ("baseline") and throughout each ECIG use period (three values in total). Data for each topography measure were averaged for each ECIG use period, with the exception of puff number.

All data analyses were performed with analysis of variance (ANOVA) using IBM SPSS (Version 27). Specifically, for plasma nicotine concentration and subjective measures that were administered before and after ECIG use, ANOVAs involved three within-subjects factors: liquid nicotine concentration (three levels: 10, 15, 30 mg/ml), device power (two levels: 15, 30 W), and time (four levels: pre-directed, post-directed, pre-*ad lib*, post-*ad lib*). For HR, the liquid concentration and power factors are the same, but the time factor had three levels (baseline, during directed use, and during *ad lib* use). For topography and subjective measures administered after ECIG use (i.e., the direct effects of ECIG use and gLMS), the liquid concentration and power factors are the same and there was no time factor (i.e., observations during or after the directed and *ad lib* use periods were analyzed separately). Additionally, because the sample involved exclusive cigarette smokers and exclusive ECIG users (see *Participant Characteristics* above, and Table 1), there is a potential that ECIG experience may have influenced each study outcome. Thus, group (two levels: cigarette smokers, ECIG users) was included as a between-subject factor in all analyses. In cases where no significant main effect or interactions including the group factor were observed, data were collapsed across group and reanalyzed using a three factor (for measures involving time as a factor) or two factor (for measures that did not involve time as a factor; see above) completely within-subjects analysis (i.e., without the group factor). In order to analyze mean differences across and within factors (liquid nicotine concentration, device power, and/or time), paired samples t-tests were used. Bonferroni corrections were used when appropriate (i.e., for non-orthogonal comparisons).

Results

For outcomes administered pre- and post-ECIG use, the results of particular interest are those that involve changes from baseline that occurred after ECIG use (either directed or *ad lib*). For this reason, the main effect of time and interactions involving the time factor are most relevant and are described below; where applicable, significant interactions with the between-subject group factor are also reported. Table 2 displays ANOVA results for the main effect of Time and all possible interactions that involve the Time factor.

For outcomes not involving time as a factor (i.e., those administered only after ECIG use), each ECIG use period was analyzed separately and Tables 3, 6, 7, and 8 display these ANOVA results.

Table 2. Statistical Analysis Results for Four-Factor Mixed Analysis of Variance (Directed + *ad libitum* use periods).

Outcome Measure	Time		Nicotine* Time		Power* Time		Time* Group		Nicotine* Time* Group		Power* Time* Group		Nicotine* Power* Time		Nicotine* Power* Time* Group	
	F	η_p^2	F	η_p^2	F	η_p^2	F	η_p^2	F	η_p^2	F	η_p^2	F	η_p^2	F	η_p^2
Plasma Nicotine [a]	**26.1**	**.58**	**4.6**	**.20**	**3.9**	**.17**	3.0	.14	1.6	.08	0.8	.04	1.7	.08	1.1	.05
HR [b]	**57.0**	**.75**	**7.6**	**.29**	**11.6**	**.38**	1.1	.06	0.8	.04	0.4	.02	1.5	.07	1.4	.07
HH [a]	F	η_p^2	F	η_p^2	F	η_p^2	F	η_p^2	F	η_p^2	F	η_p^2	F	η_p^2	F	η_p^2
Anxious	**28.5**	**.60**	0.7	.04	0.4	.02	**3.8**	**.17**	0.9	.05	0.4	.02	2.3	.11	0.1	.01
Craving	**45.4**	**.71**	**2.5**	**.11**	2.2	.10	0.5	.03	1.2	.06	0.7	.04	1.5	.07	0.8	.04
Depression	2.9	.13	0.2	.01	0.3	.02	1.1	.05	0.6	.03	0.6	.03	1.5	.07	0.7	.03
Difficulty Concentrating	**13.5**	**.42**	0.8	.04	1.4	.07	0.8	.04	0.4	.02	**5.7**	**.23**	1.0	.05	1.0	.05
Drowsy	**7.5**	**.28**	**2.3**	**.11**	0.5	.02	0.8	.04	**3.5**	**.16**	0.6	.03	0.4	.02	1.4	.07
Hunger	**5.7**	**.23**	**2.4**	**.11**	**3.1**	**.14**	0.1	.01	1.1	.06	2.4	.11	2.5	.12	1.9	.09
Impatient	**13.4**	**.41**	1.6	.08	0.4	.02	1.7	.08	1.0	.05	0.4	.02	2.0	.09	0.5	.02
Irritable	**32.4**	**.63**	0.5	.02	1.0	.05	**4.8**	**.20**	0.9	.05	0.0	.00	1.6	.08	0.4	.02
Restless	**8.9**	**.32**	0.9	.05	0.5	.03	0.6	.03	0.6	.03	1.8	.09	0.6	.03	1.1	.06
Sweets	2.7	.13	1.3	.06	0.3	.01	1.6	.08	1.4	.07	1.6	.08	0.3	.02	1.4	.07
Urge	**54.3**	**.74**	1.2	.06	0.5	.03	0.3	.02	1.1	.05	0.9	.05	0.7	.03	0.5	.03
DE Nicotine [a]	F	η_p^2	F	η_p^2	F	η_p^2	F	η_p^2	F	η_p^2	F	η_p^2	F	η_p^2	F	η_p^2
Confused	2.4	.11	0.1	.01	0.3	.02	0.2	.01	1.1	.05	**3.5**	**.16**	0.9	.05	1.0	.05
Dizzy	**7.2**	**.28**	1.5	.07	**5.3**	**.22**	0.0	.00	0.3	.02	1.6	.08	0.5	.03	0.5	.02
Headache	1.6	.08	0.6	.03	0.6	.03	0.3	.02	0.9	.05	1.8	.09	1.1	.06	1.4	.07
Heart Pounding	**4.9**	**.21**	0.7	.04	**3.8**	**.17**	1.3	.07	**2.8**	**.13**	1.2	.06	1.0	.05	**3.5**	**.16**
Lightheaded	**11.9**	**.39**	1.6	.08	**4.3**	**.19**	1.0	.05	1.9	.09	**4.4**	**.19**	1.0	.05	0.8	.04
Nauseous	2.6	.12	1.5	.08	**4.5**	**.19**	2.9	.13	0.7	.04	1.3	.06	0.8	.04	2.0	.09
Nervous	**4.7**	**.20**	0.4	.02	0.9	.05	0.6	.03	0.7	.04	1.8	.09	0.6	.03	0.5	.03
Salivation	0.5	.02	0.9	.04	0.1	.01	0.7	.03	0.5	.03	0.9	.05	0.3	.01	1.8	.09
Sweaty	0.7	.03	0.7	.04	0.2	.01	0.5	.03	0.5	.03	1.0	.05	1.6	.08	0.8	.04
Weak	2.6	.12	0.9	.05	1.2	.06	0.3	.01	2.1	.10	0.4	.02	**2.5**	**.12**	0.9	.05

[a] df T=(3,57); df N*T=(6,114); df P*T=(3,57); df T*G=(3,57); df N*T*G=(6,114); df P*T*G=(3,57); df N*P*T=(6,114); df N*P*T*G=(6,114)

[b] df T=(2,38); df N*T=(4,76); df P*T=(2,38); df T*G=(2,38); df N*T*G=(4,76); df P*T*G=(2,38); df N*P*T=(4,76); df N*P*T*G=(4,76)

Physiological Measures

Plasma Nicotine Concentration

As displayed in Table 2, no statistically significant interactions including the group factor (cigarette smokers, ECIG users) were observed, therefore plasma nicotine data were re-analyzed using a three factor completely within-subject ANOVA (i.e., with no group factor) and those results are reported here.

Significant interactions were observed for liquid nicotine concentration by time [$F(6,120)=4.7$, $p<0.05$] and device power by time [$F(3,60)=3.9$, $p<0.05$]. The means (± 1 SEM) for all conditions across time are depicted in Figure 2 Panels A (15 W) and B (30 W). As the figure shows, pre-directed use period plasma nicotine concentration means in all conditions were low and did not differ significantly by condition. However, mean plasma nicotine concentration increased significantly over time for each liquid nicotine concentration level (i.e., 10, 15, 30 mg/ml) at each power setting (i.e., 15, 30 W) after the directed use period, [$ts(20)>5.4$, $ps<0.05$]. For example, at 15 W for the 10 mg/ml condition, pre-directed mean plasma nicotine concentration was 2.1 ng/ml (SEM=0.1) and increased to 11.2 (SEM=1.7; $p<0.05$); at 15 W for the 15 mg/ml condition, mean plasma nicotine concentration was 2.1 (SEM=0.1) and increased to 8.9 (SEM=1.3; $p<0.05$); at 15 W for the 30 mg/ml condition, mean plasma nicotine concentration was 2.1 (SEM=0.1) and increased to 16.1 (SEM=2.8; $p<0.05$; See Figure 2, Panel A). Similarly, at 30 W for the 10 mg/ml condition, pre-directed mean plasma nicotine concentration was 2.2 (SEM=0.1) and increased to 13.9 (SEM=2.1; $p<0.05$); at 30 W for the 15 mg/ml condition, mean plasma nicotine concentration was 2.0 (SEM=0.02) and increased to 15.2

(2.8; $p<0.05$); at 30 W for the 30 mg/ml condition, mean plasma nicotine concentration was 2.2 (SEM=0.1) and increased to 21.4 (SEM=4.2; $p<0.05$; See Figure 2, Panel B).

Mean plasma nicotine concentration was significantly greater in the 15 mg/ml liquid nicotine condition at 30 W (M=15.2, SEM=2.8) when compared to 15 W [(M=8.9, SEM=1.3); $t(20)=-3.4$, $p<.05$] after the directed use period. No significant differences were observed across liquid nicotine concentration [ps>0.025] following the directed use period.

Following the *ad lib* use period, mean plasma nicotine concentration increased significantly over time for each liquid nicotine concentration level at each power setting [ts(20)>5.1, ps<0.05]. For example, at 15 W for the 10 mg/ml condition, pre-*ad lib* use period mean plasma nicotine concentration was 4.5 (SEM=0.5) and increased significantly to 16.7 (SEM=2.7; $p<.05$); at 15 W for the 15 mg/ml condition, mean plasma nicotine concentration was 4.6 (SEM=0.5) and increased to 15.4 (SEM=3.3; $p<0.05$); at 15 W for the 30 mg/ml condition, mean plasma nicotine concentration was 5.9 (SEM=0.7) and increased to 19.4 (SEM=3.3; $p<0.05$; See Figure 2, Panel A). Similarly, at 30 W for the 10 mg/ml condition, mean plasma nicotine concentration was 6.6 (SEM=1.0) and increased to 17.4 (SEM=3.0; $p<0.05$); at 30 W for the 15 mg/ml condition, mean plasma nicotine concentration was 7.1 (SEM=0.8) and increased to 20.4 (SEM=3.7; $p<0.05$); at 30 W for the 30 mg/ml condition, mean plasma nicotine concentration was 9.0 (SEM=1.0) and increased to 16.2 (SEM=2.8; $p<0.05$; See Figure 2, Panel B).

No significant differences were observed after the *ad lib* use period across liquid nicotine concentration [ps>0.025] or device power setting [ps>0.05].

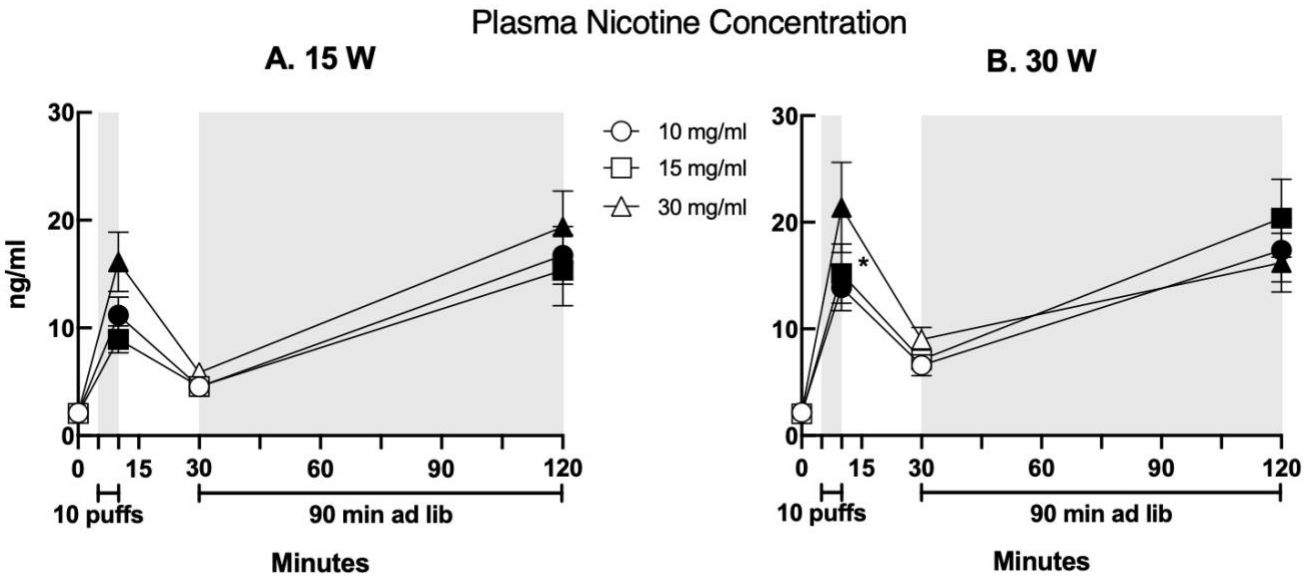

Figure 2. Mean data (± SEM) for plasma nicotine across conditions (N=21). Participants completed a directed, 10-puff use period and a 90-minute *ad libitum* use period (use periods shaded gray) in six conditions based on liquid nicotine concentration condition (10 mg/ml, circle symbol; 15 mg/ml, square symbol; 30 mg/ml, triangle symbol) and device power setting (15 W, Panel A; 30 W Panel B). Filled symbols indicate a significant difference from pre-bout plasma nicotine concentration (same condition). Number sign (#) indicates significant difference from 10 mg at same time point (none observed in the current figure). Asterisk (*) indicates significant difference from 15 W at same time point. Carat (^) indicates significant difference from ECIG users at same condition and timepoint (none observed in the current figure). All *p*'s <0.05; paired samples t-tests.

Heart Rate

For heart rate (HR), significant interactions were observed for liquid nicotine concentration by time and device power by time; no significant main effect of group or significant interactions involving the group factor were observed. Therefore, HR data was re-analyzed using a three factor completely within-subject ANOVA (i.e., with no group factor), and those results are reported here.

Significant interactions were observed for liquid nicotine concentration by time [$F(4,80)=7.7$, $p<0.05$] and device power by time [$F(2,40)=12.1$, $p<0.05$]. The means (± 1 SEM) for all conditions across time are depicted in Figure 3 Panels A (15 W) and B (30 W). Similar to plasma nicotine concentration, HR increased significantly over time for each liquid nicotine concentration level (i.e., 10, 15, 30 mg/ml) at each power setting (i.e., 15, 30 W) during the directed use period, [$ts(20)<-6.4$, $ps<0.05$]. For example, at 15 W for the 10 mg/ml condition, baseline mean HR was 65.5 BPM (SEM=1.4) and increased to 76.6 (SEM=2.1; $p<0.05$); at 15 W for the 15 mg/ml condition, baseline mean HR was 64.7 BPM (SEM=2.1) and increased to 75.9 (SEM=3.0; $p<0.05$); at 15 W for the 30 mg/ml condition, baseline mean HR was 64.8 BPM (SEM=1.8) and increased to 78.4 (SEM=2.6; $p<0.05$). Similarly, at 30 W for the 10 mg/ml condition, baseline mean HR was 65.8 BPM (SEM=1.9) and increased to 78.9 (SEM=2.8; $p<0.05$); at 30 W for the 15 mg/ml condition, baseline mean HR was 64.5 BPM (SEM=2.0) and increased to 78.4 (SEM=2.5; $p<0.05$); at 30 W for the 30 mg/ml condition, baseline mean HR was 64.0 (SEM=1.9) and increased to 83.3 (SEM=3.0; $p<0.05$).

Also, significant differences across liquid nicotine concentration and device power setting were observed during the directed use period. Specifically, mean HR was greater in the 30 mg/ml;30 W condition (M=83.3, SEM=3.0) when compared to the 10 mg/ml;30 W condition

[(M=78.9, SEM=2.8); $t(20)=-2.7$, $p<0.025$]. Additionally, mean HR was significantly different when comparing the 30 mg/ml liquid nicotine condition at 15 W (M=78.4, SEM=2.6) and 30 W [(M=83.3, SEM=3.0); $t(20)=-2.5$, $p<0.05$].

During the *ad lib* use period, mean HR increased significantly over time for each liquid nicotine concentration level at each power setting [$ts(20)<-4.1$, $ps<0.025$]. For example, at 15 W for the 10 mg/ml condition, baseline mean HR was 65.5 BPM (SEM=1.4) and increased significantly to 73.4 (SEM=2.3; $p<0.025$); at 15 W for the 15 mg/ml condition, mean HR was 64.7 BPM (SEM=2.1) and increased to 72.0 (SEM=2.7; $p<0.025$); at 15 W for the 30 mg/ml condition, mean HR was 64.8 BPM (SEM=1.8) and increased to 73.0 (SEM=2.2; $p<0.025$; See Figure 3, Panel A). Similarly, at 30 W for the 10 mg/ml condition, baseline mean HR was 65.8 BPM (SEM=1.9) and increased to 73.8 BPM (SEM=2.6; $p<0.025$); at 30 W for the 15 mg/ml condition, mean HR was 64.5 BPM (SEM=2.0) and increased to 74.0 (SEM=2.1; $p<0.025$); at 30 W for the 30 mg/ml condition, mean HR was 64.0 (SEM=1.9) and increased to 72.6 (SEM=2.4; $p<0.025$; See Figure 3, Panel B).

No significant differences were observed during the *ad lib* use period across liquid nicotine conditions [$ps>0.025$] or device power settings [$ps>0.05$].

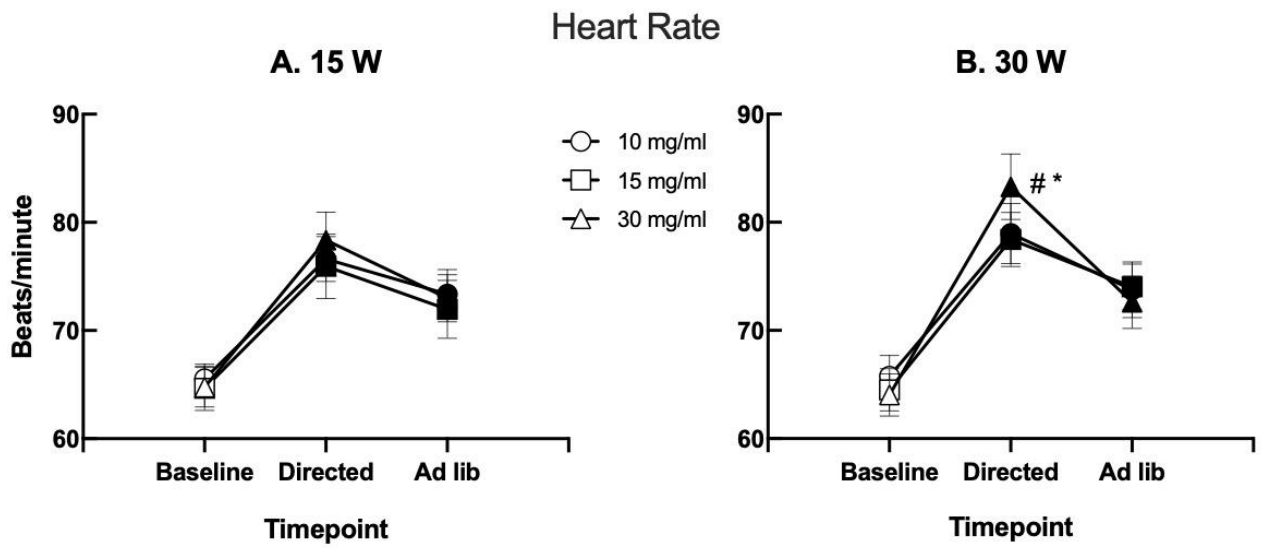

Figure 3. Mean data (± SEM) for HR across conditions (N=21).
In all other respects, the figure is identical to Figure 2.

Puff Topography

Topography observations occurred during ECIG use only. Therefore, the factor of time was not included in analysis and the directed and *ad lib* use periods were analyzed separately. The liquid concentration, power, and group factors are the same as all other measures. As displayed in Table 3, statistically significant interactions including the group factor were observed in items Puff Count, Duration, and Volume during the *ad lib* period only. No significant interactions including the group factor were observed during the directed use period for any topography items, nor during the *ad lib* use period for IPI and AFR. Therefore, the directed use period for all items, as well as IPI and AFR (both use periods) were re-analyzed using a two factor completely within-subject ANOVA (i.e., with no group factor), and these results are reported here. Significant group interactions are reported when appropriate. The means (SEM) for all conditions in each topography measure are displayed in Table 4. Group differences in puff count, puff volume, and puff duration during the *ad lib* use period are displayed in Table 5.

Table 3. Statistical Analysis Results for Three-Factor Mixed Analysis of Variance (Directed + *ad libitum* use periods).

	Nicotine		**Power**		**Nicotine * Power**		**Nicotine * Group**		**Power * Group**		**Nicotine * Power * Group**	
Topography	F	η_p^2	F	η_p^2	F	η_p^2	F	η_p^2	F	η_p^2	F	η_p^2
Puff Count												
Directed [a]	2.5	.13	**4.6**	**.21**	1.2	.06	0.3	.02	2.4	.12	2.3	.12
Ad libitum [b]	3.5	.18	2.3	.13	0.3	.02	1.0	.06	**4.8**	**.23**	0.9	.05
Puff Duration [a]												
Directed	**22.7**	**.57**	**85.6**	**.83**	2.3	.12	1.9	.10	1.2	.07	1.3	.07
Ad libitum	**9.8**	**.38**	**76.9**	**.83**	**4.1**	**.21**	2.3	.12	**4.9**	**.23**	2.8	.15
Puff Volume												
Directed [a]	**9.9**	**.37**	**38.4**	**.69**	2.6	.13	1.0	.06	1.2	.07	0.3	.02
Ad libitum [b]	**5.6**	**.26**	**35.4**	**.69**	1.3	.07	0.5	.03	**6.1**	**.28**	0.6	.04
IPI												
Directed [a]	2.5	.13	**19.7**	**.54**	1.1	.06	0.1	.00	0.2	.01	2.2	.12
Ad libitum [b]	**6.5**	**.29**	3.2	.17	3.6	.18	1.7	.09	0.9	.06	2.2	.12
AFR												
Directed [a]	0.1	.01	2.7	.14	0.3	.02	0.0	.00	1.4	.08	0.0	.00
Ad libitum [b]	1.0	.06	2.1	.12	0.1	.01	0.0	.00	0.5	.03	0.2	.02

[a] df N=(2,34); df P=(1,17); df N*P=(2,34); df N*G=(2,34); df P*G=(1,17); df N*P*G=(2,34)

[b] df N=(2,32); df P=(1,16); df N*P=(2,32); df N*G=(2,32); df P*G=(1,16); df N*P*G=(2,32)

Table 4. Mean (SEM) Puff Topography by Liquid Nicotine Concentration and Device Power (Directed + *ad libitum* use periods).

	10 mg		15 mg		30 mg	
	15 W	30 W	15 W	30 W	15 W	30 W
Puff count						
Directed [a]	10.1 (.1)	9.9 (.1)	10.5 (.3)	10.1 (.1)	10.0 (.1)	9.9 (.1)
Ad libitum [b]	50.1 (12.9)	44.1 (8.3)	44.7 (5.6)	38.5 (7.2)	35.4 (7.2)	34.6 (11.0)
Puff duration (sec)						
Directed [a]	3.73 (.30)	**2.50 (.19)***	**3.21 (.22)#**	**2.12 (.14)*#**	**2.87 (.19)#**	**2.04 (.19)*#**
Ad libitum [b]	3.81 (.41)	2.20 (.21)	3.28 (.30)	1.93 (.16)	2.99 (.24)	1.93 (.22)
Puff volume (ml)						
Directed [a]	667.8 (94.4)	**410.5 (57.2)***	**537.0 (68.3)#**	**346.2 (47.2)*#**	**464.5 (56.8)#**	**316.0 (47.9)*#**
Ad libitum [b]	600.0 (103.0)	326.9 (58.5)	536.1 (93.0)	298.5 (54.3)	459.4 (75.4)	267.1 (45.8)
Inter-puff Interval						
Directed [a]	25.8 (.3)	**27.5 (.3)***	26.1 (.7)	27.9 (.4)	**27.1 (.4)#**	27.8 (.3)
Ad libitum [b]	184.7 (47.3)	130.3 (11.8)	126.8 (13.2)	**171.9 (24.0)#**	187.0 (23.6)	**298.0 (65.0)*#**
Flow rate (ml/sec)						
Directed [a]	172.6 (16.7)	156.0 (14.7)	162.7 (14.7)	156.2 (16.0)	172.1 (24.6)	149.4 (16.0)
Ad libitum [b]	160.5 (21.0)	145.4 (15.7)	149.9 (16.0)	140.8 (16.9)	146.1 (16.1)	129.3 (15.0)

[a] n=19

[b] n=18

Note: Asterisk (*) indicates significant difference from 15 W at same time point. Number sign (#) indicates significant difference from 10 mg at same time point.

Table 5. Mean (SEM) Puff Topography by Group, Liquid Nicotine Concentration, and Device Power (*ad libitum* use period).

	10 mg		15 mg		30 mg	
	15 W	30 W	15 W	30 W	15 W	30 W
Count						
ECIG users [c]	44.0 (9.1)	37.7 (6.1)	45.7 (8.3)	**31.6 (5.8)***	31.2 (4.6)	**20.0 (5.4)*#**
Smokers [c]	56.1 (24.8)	50.4 (15.6)	43.7 (8.0)	45.4 (13.2)	39.6 (14.0)	49.1 (20.9)
Duration (sec)						
ECIG users [c]	4.85 (.49)	**2.70 (.28)***	**4.04 (.36)#**	**2.30 (.20)*#**	**3.53 (.28)#**	**2.39 (.35)***
Smokers [c]	**2.78 (.46)^**	**1.71 (.21)^***	**2.53 (.34)^**	**1.55 (.18)^***	**2.44 (.30)^**	**1.47 (.15)^***
Volume (ml)						
ECIG users [c]	836.7 (157.5)	**438.8 (94.8)***	753.0 (147.7)	**419.4 (85.1)***	640.3 (104.5)	**377.3 (70.9)***
Smokers [c]	**363.4 (79.4)^**	**215.1 (49.4)^***	**319.3 (56.5)^**	**177.6 (40.4)^*#**	**278.4 (71.0)^**	**156.9 (29.1)^**

[c] n=9

Note: Carat (^) indicates significant difference from ECIG user group within the same condition. Asterisk (*) indicates significant difference from 15 W within the same group and liquid nicotine concentration condition. Number sign (#) indicates significant difference from 10 mg within the same group and device power setting.

Puff Count. A significant main effect of power was observed during the directed use period (see Table 3); no significant main effect of group or significant interactions involving the group factor were observed for puff count during the directed use period. Therefore, puff count during the directed use period was re-analyzed using a two factor completely within-subject ANOVA (i.e., with no group factor), and no main effects or interactions were observed. During the *ad lib* use period, a significant interaction was observed for power by group (see Table 3). The means for puff count in all conditions collapsed across group are displayed in Table 4. As displayed in the table, puff count did not differ during the directed use period, as expected. The means for puff count by group and condition during the *ad lib* use period are displayed in Table 5. As displayed in the table, puff count decreased as liquid nicotine concentration and device power setting increased during the *ad lib* use period.

Significant differences in puff count during the *ad lib* use period were revealed in the ECIG user group only (see Table 5). Specifically, in the 30 W condition, mean puff count was significantly greater in the 10 mg/ml condition (M=37.7, SEM=6.1) when compared to the 30 mg/ml condition [(M=20.0, SEM=5.4); $t(8)=5.6$, $p<0.025$]. In the 15 mg/ml condition, mean puff count was significantly greater in the 15 W condition (M=45.7, SEM=8.3) when compared to the 30 W condition [(M=31.6, SEM=5.8); $t(8)=2.7$, $p<0.05$]. Additionally, in the 30 mg/ml condition, mean puff count was significantly greater in the 15 W condition (M=31.2, SEM=4.6) when compared to the 30 W condition [(M=20.0, SEM=5.4); $t(8)=5.0$, $p<0.05$] during the *ad lib* use period (see Table 5).

No significant differences in puff count were observed across liquid nicotine concentration [*p*s>0.025] or device power setting [*p*s>0.05] during the directed or *ad lib* use periods in the cigarette smoker group.

Puff Duration. Significant main effects of device power and liquid nicotine concentration were observed for puff duration during the directed use period (see Table 3); no significant main effect of group or significant interactions involving the group factor were observed for puff duration during the directed use period. Therefore, puff duration during the directed use period was re-analyzed using a two factor completely within-subject ANOVA (i.e., with no group factor), and significant main effects of device power [$F(1,18)=85.8$, $p<0.05$] and liquid nicotine concentration [$F(2,36)=22.3$, $p<0.05$] were observed. During the *ad lib* use period, a significant interaction was observed for power by group, as well as a significant interaction for nicotine by power (see Table 3). Additionally, main effects of device power and liquid nicotine concentration were observed during the *ad lib* use period (see Table 3). The means for puff duration in all conditions collapsed across group are displayed in Table 4. As the table shows, puff duration decreased as liquid nicotine concentration and device power setting increased. The means for puff duration by group and condition during the *ad lib* use period are displayed in Table 5.

Significant differences in puff duration across power setting and liquid nicotine concentration were observed during the directed use period. Specifically, in the 10 mg/ml condition, mean puff duration was significantly greater in the 15 W condition (M=3.7, SEM=0.3) when compared to the 30 W condition [(M=2.5, SEM=0.2); $t(18)=6.8$, $p<0.05$]. Similarly, within the 15 mg/ml condition, mean puff duration was significantly greater in the 15 W condition (M=3.2, SEM=0.2) when compared to the 30 W condition [(M=2.1, SEM=0.1); $t(18)=8.2$, $p<0.05$]; within the 30 mg/ml condition, mean puff duration was significantly greater in the 15 W condition (M=2.9, SEM=0.2) when compared to the 30 W condition [(M=2.0, SEM=0.2); $t(18)=5.6$, $p<0.05$]. Comparing across liquid nicotine concentration conditions, within the 15 W

condition, mean puff duration was significantly greater in the 10 mg/ml condition (M=3.7, SEM=0.3) when compared to the 15 mg/ml condition [(M=3.2, SEM=0.2); $t(18)=3.0$, $p<0.025$], and when compared to the 30 mg/ml condition [(M=2.9, SEM=0.2); $t(18)=4.5$, $p<0.025$]. Similarly, within the 30 W condition, mean puff duration was significantly greater in the 10 mg/ml condition (M=2.5, SEM=0.2) when compared to the 15 mg/ml condition [(M=2.1, SEM=0.1); $t(18)=3.6$, $p<0.025$], as well as to the 30 mg/ml condition [(M=2.0, SEM=0.2); $t(18)=5.0$, $p<0.025$].

During the *ad lib* use period, significant differences were observed between groups in all conditions [$ts(16)>2.2$, $p<0.05$]. Within the ECIG user group, significant differences between power setting and liquid nicotine concentration conditions were observed during the *ad lib* use period (see Table 5). Specifically, in the 10 mg/ml condition, mean puff duration was significantly greater in the 15 W condition (M=4.9, SEM=0.5) when compared to the 30 W condition [(M=2,7, SEM=0.3); $t(8)=6.6$, $p<0.05$]. Similarly, within the 15 mg/ml condition, mean puff duration was significantly greater in the 15 W condition (M=4.0, SEM=0.4) when compared to the 30 W condition [(M=2.3, SEM=0.2); $t(8)=6.6$, $p<0.05$]; within the 30 mg/ml condition, mean puff duration was significantly greater in the 15 W condition (M=3.5, SEM=0.3) when compared to the 30 W condition [(M=2.4, SEM=0.6); $t(8)=4.4$, $p<0.05$]. Comparing across liquid nicotine concentration conditions, within the 15 W condition, mean puff duration was significantly greater in the 10 mg/ml condition (M=4.9, SEM=0.5) when compared to the 15 mg/ml condition [(M=4.0, SEM=0.4); $t(8)=3.1$, $p<0.025$], and when compared to the 30 mg/ml condition [(M=3.5, SEM=0.3); $t(8)=4.7$, $p<0.025$]. Similarly, within the 30 W condition, mean puff duration was significantly greater in the 10 mg/ml condition (M=2.7, SEM=0.3) when compared to the 15 mg/ml condition [(M=2.3, SEM=0.2); $t(8)=3.1$, $p<0.025$].

Within the cigarette smoker group, significant differences across power setting conditions were observed during the *ad lib* use period (see Table 5). Specifically, in the 10 mg/ml condition, mean puff duration was significantly greater in the 15 W condition (M=2.8, SEM=0.5) when compared to the 30 W condition [(M=1.7, SEM=0.2); $t(8)=3.4$, $p<0.05$]. Similarly, within the 15 mg/ml condition, mean puff duration was significantly greater in the 15 W condition (M=2.5, SEM=0.3) when compared to the 30 W condition [(M=1.6, SEM=0.2); $t(8)=4.8$, $p<0.05$]; within the 30 mg/ml condition, mean puff duration was significantly greater in the 15 W condition (M=2.4, SEM=0.3) when compared to the 30 W condition [(M=1.5, SEM=0.2); $t(8)=4.4$, $p<0.05$].

Puff Volume. Significant main effects of device power and liquid nicotine concentration were observed for puff volume during the directed use period (see Table 3); no significant main effect of group or significant interactions involving the group factor were observed for puff volume during the directed use period. Therefore, puff volume during the directed use period was re-analyzed using a two factor completely within-subject ANOVA (i.e., with no group factor), and significant main effects of device power [$F(1,18)=38.8$, $p<0.05$] and liquid nicotine concentration [$F(2,36)=10.3$, $p<0.05$] were observed. During the *ad lib* use period, a significant interaction was observed for power by group, as well as main effects of device power and liquid nicotine concentration (see Table 3). The means for puff volume in all conditions collapsed across group are displayed in Table 4. As the table shows, puff volume decreased as liquid nicotine concentration and device power setting increased. The means for puff volume by group and condition during the *ad lib* use period are displayed in Table 5.

Significant differences across power setting and liquid nicotine concentration conditions were observed during the directed use period. Specifically, in the 10 mg/ml condition, mean puff

volume was significantly greater in the 15 W condition (M=667.8, SEM=94.4) when compared to the 30 W condition [(M=410.5, SEM=57.2); $t(18)=5.0$, $p<0.05$]. Similarly, within the 15 mg/ml condition, mean puff volume was significantly greater in the 15 W condition (M=537.0, SEM=68.3) when compared to the 30 W condition [(M=346.2, SEM=47.2); $t(18)=5.8$, $p<0.05$]; within the 30 mg/ml condition, mean puff volume was significantly greater in the 15 W condition (M=464.5, SEM=56.8) when compared to the 30 W condition [(M=316.0, SEM=47.9); $t(18)=3.9$, $p<0.05$]. Comparing across liquid nicotine concentration conditions, within the 15 W condition, mean puff volume was significantly greater in the 10 mg/ml condition (M=667.8, SEM=94.4) when compared to the 15 mg/ml condition [(M=537.0, SEM=68.3); $t(18)=2.9$, $p<0.025$], and when compared to the 30 mg/ml condition [(M=464.5, SEM=56.8); $t(18)=3.1$, $p<0.025$]. Additionally, within the 30 W condition, mean puff volume was significantly greater in the 10 mg/ml condition (M=410.5, SEM=57.2) when compared to the 15 mg/ml condition [(M=346.2, SEM=47.2); $t(18)=2.8$, $p<0.025$], as well as to the 30 mg/ml condition [(M=316.0, SEM=47.9); $t(18)=4.0$, $p<0.025$].

During the *ad lib* use period, independent samples t-tests revealed significant differences between groups in all conditions [$ts(16)>2.0$, $p<0.05$]. Within the ECIG user group, significant differences between power setting condition were observed during the *ad lib* use period (see Table 5). Specifically, in the 10 mg/ml condition, mean puff volume was significantly greater in the 15 W condition (M=836.7, SEM=157.5) when compared to the 30 W condition [(M=438.8, SEM=94.8); $t(8)=3.9$, $p<0.05$]. Similarly, within the 15 mg/ml condition, mean puff volume was significantly greater in the 15 W condition (M=753.0, SEM=147.7) when compared to the 30 W condition [(M=419.4, SEM=85.1); $t(8)=4.3$, $p<0.05$]; within the 30 mg/ml condition, mean puff

volume was significantly greater in the 15 W condition (M=640.3, SEM=104.5) when compared to the 30 W condition [(M=377.3, SEM=70.9); $t(8)=4.7$, $p<0.05$].

Within the cigarette smoker group, significant differences across power setting and liquid nicotine concentration conditions were observed during the *ad lib* use period (see Table 5). Specifically, in the 10 mg/ml condition, mean puff volume was significantly greater in the 15 W condition (M=363.4, SEM=79.4) when compared to the 30 W condition [(M=215.1, SEM=49.4); $t(8)=2.3$, $p<0.05$]. Similarly, within the 15 mg/ml condition, mean puff volume was significantly greater in the 15 W condition (M=319.3, SEM=56.5) when compared to the 30 W condition [(M=177.6, SEM=40.4); $t(8)=3.7$, $p<0.05$]. Comparing across liquid nicotine concentration conditions, within the 30 W condition, mean puff volume was significantly greater in the 10 mg/ml condition (M=215.1, SEM=49.4) when compared to the 15 mg/ml condition [(M=177.6, SEM=40.4); $t(8)=3.0$, $p<0.025$].

Inter-puff Interval (IPI). A significant main effect of device power was observed for IPI during the directed use period (see Table 3); no significant main effect of group or significant interactions involving the group factor were observed for IPI during the directed use period. Therefore, IPI during the directed use period was re-analyzed using a two factor completely within-subject ANOVA (i.e., with no group factor), and a significant main effect of device power [$F(1,18)=20.4$, $p<0.05$] was observed. During the *ad lib* use period, a significant main effect of liquid nicotine concentration was observed (see Table 3); as in the directed use period, no significant main effect of group or significant interactions involving the group factor were observed for IPI during the *ad lib* use period. Therefore, IPI during the *ad lib* use period was re-analyzed using a two factor completely within-subject ANOVA (i.e., with no group factor), and a significant main effect of liquid nicotine concentration [$F(2,34)=6.2$, $p<0.05$] was observed. The

means for IPI in all conditions collapsed across group are displayed in Table 4. As the table shows, IPI decreased as liquid nicotine concentration and device power setting increased.

Significant differences across power setting and liquid nicotine concentration conditions were observed during the directed use period. Specifically, in the 10 mg/ml condition, mean IPI was significantly greater in the 15 W condition (M=25.8, SEM=0.3) when compared to the 30 W condition [(M=27.5, SEM=0.3); $t(18)=-6.0$, $p<0.05$]. Comparing across liquid nicotine concentration conditions, within the 15 W condition, mean IPI was significantly greater in the 10 mg/ml condition (M=25.8, SEM=0.3) when compared to the 30 mg/ml condition [(M=27.1, SEM=0.4); $t(18)=-2.9$, $p<0.025$].

During the *ad lib* use period, a significant difference across liquid nicotine concentration conditions was observed. Specifically, in the 30 W condition, mean IPI was significantly greater in the 10 mg/ml condition (M=130.3, SEM=11.8) when compared to the 30 mg/ml condition [(M=298.0, SEM=65.0); $t(17)=-2.7$, $p<0.025$].

Subjective Effects

Hughes-Hatsukami

Statistically significant interactions including the group factor were observed in the following items: Anxious, Difficulty Concentrating, Drowsy, and Irritable (see Table 2), and these items were analyzed using a four factor mixed (within and between subjects) ANOVA. For the remaining HH items, no significant interactions including the group factor were observed. Therefore, all remaining items were re-analyzed using a three factor completely within-subject ANOVA (i.e., with no group factor). Significant main effects of group and significant interactions including the group factor are detailed below.

Anxious. For the item "anxious", a significant interaction was observed for time by group (see Table 2). The means (± 1 SEM) for both groups across time are depicted in Figure 4 Panels A (directed use period) and B (*ad lib* use period). As the figure shows, mean anxious ratings appeared to differ by group prior to the directed use period, though independent samples t-tests did not reveal a significant difference between groups at any time point [*p*s>0.05].

Significant differences were revealed when comparing anxious ratings pre- and post-directed use period within each group. In the ECIG user group, pre-directed mean anxious ratings were 24.8 (SEM=6.7) and significantly decreased to 13.6 [(SEM=3.8); $t(10)=2.7$, $p<0.05$]. Additionally, in the cigarette smoker group, pre-directed mean anxious ratings were 39.4 (SEM=7.9) and significantly decreased to 10.8 [(SEM=3.7); $t(9)=4.4$, $p<0.05$].

A significant difference was observed within the cigarette smoker group when comparing mean anxious ratings pre- and post-*ad lib* use period. Specifically, pre-*ad lib* mean anxious ratings were 11.5 (SEM=4.2) and significantly decreased to 8.1 [(SEM=3.2); $t(9)=2.8$, $p<0.05$].

Significant differences were not observed when comparing across liquid nicotine conditions [*p*s>0.025], or device power settings [*p*s>0.05] following the *ad lib* use period.

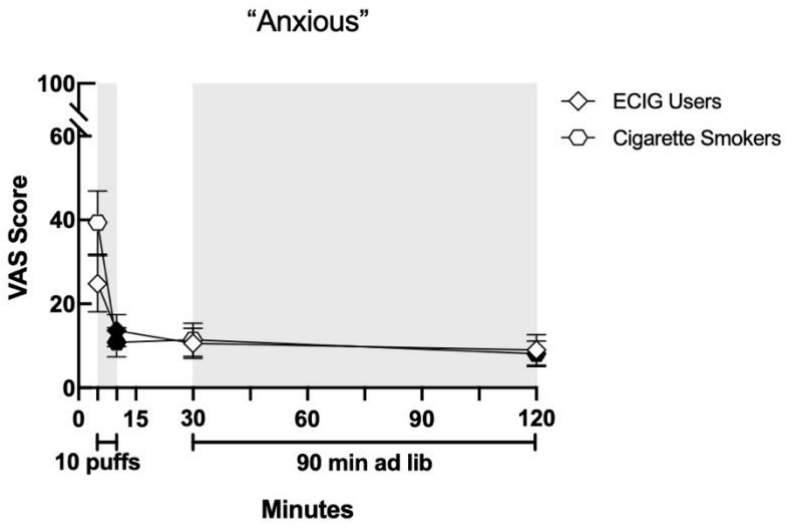

Figure 4. Mean data (± SEM) for the Hughes-Hatsukami "Anxious" item by group, collapsed across liquid nicotine concentration and device power setting (N=21).
ECIG users (n=11; diamond symbol) and cigarette smokers (n=10; hexagon symbol) completed a directed, 10-puff use period and a 90-minute *ad libitum* use period. In all other respects, the figure is identical to Figure 2.

Craving. For the item "craving an e-cigarette/cigarette/nicotine", a significant interaction was observed for liquid nicotine concentration by time (see Table 2); no significant main effect of group or significant interactions involving the group factor were observed. Therefore, "craving an e-cigarette/cigarette/nicotine" was re-analyzed using a three factor completely within-subject ANOVA (i.e., with no group factor), and a significant interaction was observed for liquid nicotine concentration by time [$F(6,120)=2.5$, $p<0.05$]. The means (± 1 SEM) for all conditions across time are depicted in Figure 5 Panels A (15 W) and B (30 W). As the figure shows, mean craving ratings did not differ significantly by condition prior to the directed use period. However, craving ratings decreased significantly over time for each liquid nicotine concentration level (i.e., 10, 15, 30 mg/ml) at each power setting (i.e., 15, 30 W) after the directed use period, [$ts(20)>4.4$, $ps<0.05$]. For example, at 15 W for the 10 mg/ml condition, pre-directed mean craving ratings were 80.7 (SEM=5.6) and significantly decreased to 55.0 (SEM=7.0; $p<0.05$); at 15 W for the 15 mg/ml condition, mean craving ratings were 79.8 (SEM=6.1) and significantly decreased to 41.0 (SEM=7.7; $p<0.05$); at 15 W for the 30 mg/ml condition, mean craving ratings were 73.8 (SEM=7.3) and significantly decreased to 41.1 (SEM=6.3; $p<0.05$). Similarly, at 30 W for the 10 mg/ml condition, pre-directed mean craving ratings were 72.4 (SEM=7.1) and significantly decreased to 41.5 (SEM=6.0; $p<0.05$); at 30 W for the 15 mg/ml condition, mean craving ratings were 70.4 (SEM=7.4) and significantly decreased to 34.3 (SEM=6.0; $p<0.05$); at 30 W for the 30 mg/ml condition, mean craving ratings were 79.0 (SEM=5.6) and significantly decreased to 27.3 (SEM=4.8; $p<0.05$).

Significant differences across liquid nicotine concentration and device power setting were observed following the directed use period. Specifically, mean craving ratings were significantly lower in the 30 mg/ml;30 W condition (M=27.3, SEM=4.8) when compared to the 10 mg/ml;30

W condition [(M=41.5, SEM=6.0); $t(20)=2.6$, $p<0.025$]. Additionally, mean craving ratings were significantly greater in the 30 mg/ml liquid nicotine condition at 15 W (M=41.1, SEM=6.2) when compared to 30 W [(M=27.3, SEM=4.8); $t(20)=2.8$, $p<0.05$].

Following the *ad lib* use period, mean craving ratings decreased significantly over time for each liquid nicotine concentration level at each power setting [ts(20)>3.4, ps<0.05]. For example, at 15 W for the 10 mg/ml condition, pre-*ad lib* mean craving ratings were 59.2 (SEM=6.0) and significantly decreased to 35.1 (SEM=6.2; $p<0.05$); at 15 W for the 15 mg/ml condition, mean craving ratings were 52.5 (SEM=6.9) and significantly decreased to 30.3 (SEM=6.5; $p<0.05$); at 15 W for the 30 mg/ml condition, mean craving ratings were 54.8 (SEM=6.0) and significantly decreased to 28.7 (SEM=6.7; $p<0.05$; See Figure 4, Panel A). Similarly, at 30 W for the 10 mg/ml condition, pre-*ad lib* mean craving ratings were 55.5 (SEM=6.2) and significantly decreased to 26.9 (SEM=6.2; $p<0.05$); at 30 W for the 15 mg/ml condition, mean craving ratings were 49.5 (SEM=6.3) and significantly decreased to 25.6 (SEM=5.9; $p<0.05$); at 30 W for the 30 mg/ml condition, mean craving ratings were 44.7 (SEM=5.4) and significantly decreased to 23.7 (SEM=5.0; $p<0.05$; See Figure 5, Panel B).

No significant differences were observed after the *ad lib* use period across liquid nicotine conditions [ps>0.025] or device power settings [ps>0.05].

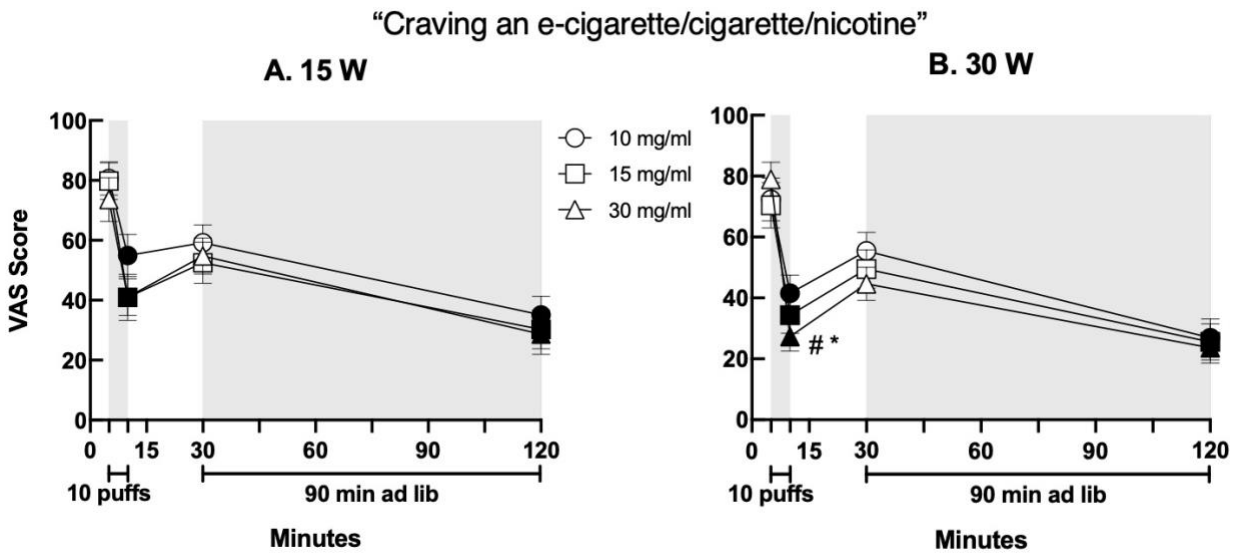

Figure 5. Mean data (± SEM) for the Hughes-Hatsukami "Craving an e-cig/cigarette/nicotine" item across conditions (N=21).
In all other respects, the figure is identical to Figure 2.

Difficulty Concentrating. For the item "difficulty concentrating", a significant three-way interaction was observed for power by time by group (see Table 2). The means (± 1 SEM) across time (collapsed across liquid nicotine concentration) are depicted in Figure 6 Panels A (directed use period) and B (*ad lib* use period). As the figure shows, mean difficulty concentrating ratings differed significantly by device power and group prior to the directed use period. Specifically, ratings of difficulty concentrating were significantly greater in the ECIG user group (M=35.7, SEM=8.2) when compared to the cigarette smoker group (M=19.3, SEM=4.5) before the directed use period in the 15 W condition [$t(19)=1.7$, $p<0.05$].

Significant differences were revealed when comparing difficulty concentrating ratings pre- and post-directed use period within each group and power condition. For example, in the ECIG user group at 15 W, pre-directed mean difficulty concentrating ratings were 35.7 (SEM=8.2) and significantly decreased to 20.2 [(SEM=5.3); $t(10)=3.1$, $p<0.05$]. Additionally, in the cigarette smoker group at 30 W, pre-directed mean difficulty concentrating ratings were 23.4 (SEM=5.7) and significantly decreased to 10.4 [(SEM=5.2); $t(9)=3.7$, $p<0.05$].

No significant differences between or within groups were observed when comparing difficulty concentrating ratings pre- and post-*ad lib* use period [$ps>0.05$], across liquid nicotine concentration [$ps>0.025$], or device power setting [$ps>0.05$].

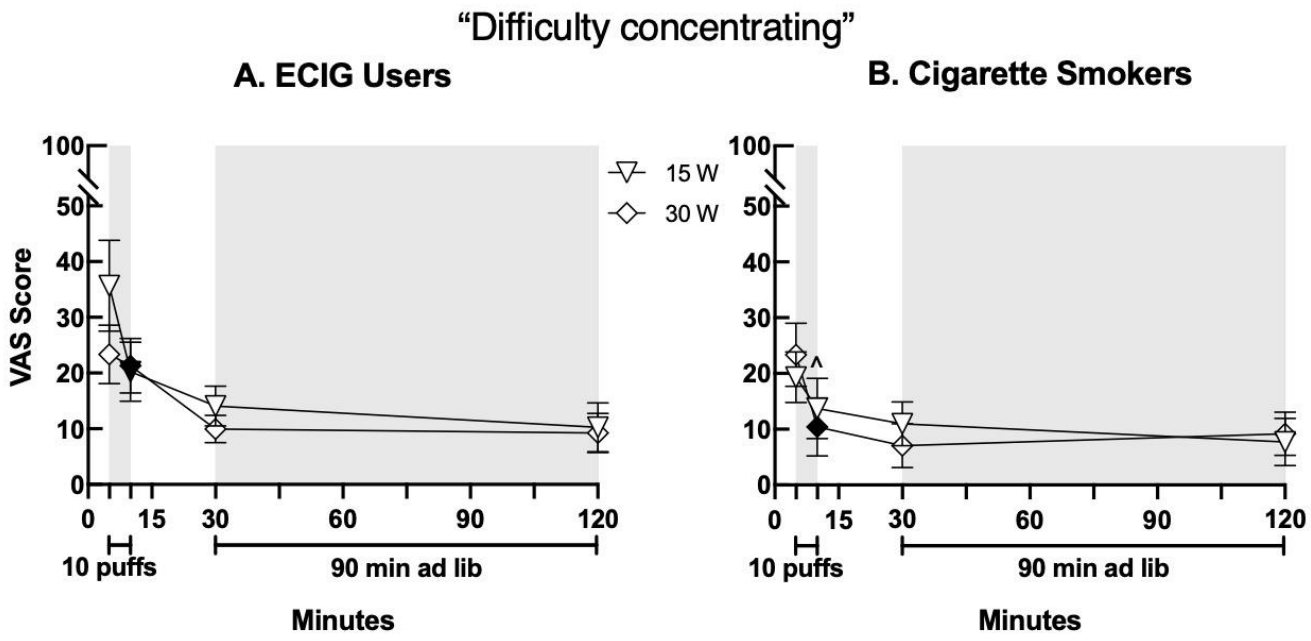

Figure 6. Mean data (± SEM) for the Hughes-Hatsukami "Difficulty concentrating" item across time by device power setting and collapsed across liquid nicotine concentration (N=21). ECIG users (n=11; Panel A) and cigarette smokers (n=10; Panel B) completed a directed, 10-puff use period and a 90-minute *ad libitum* use period based on liquid nicotine concentration condition (data are collapsed across this condition) and device power setting (15 W, inverted triangle symbol; 30 W, diamond symbol). In all other respects, the figure is identical to Figure 2.

Drowsy. For the item "drowsiness", a significant three-way interaction was observed for liquid nicotine concentration by time by group [$F(6,114)=3.5$, $p<0.05$; see Table 2]. The means (± 1 SEM) across time (collapsed across device power) are depicted in Figure 7 Panels A (directed use period) and B (*ad lib* use period). As the figure shows, mean drowsiness ratings decreased in both groups following the directed use period. However, independent samples t-tests did not reveal a significant difference between groups at any relevant timepoints [*p*s>0.05].

Significant differences were revealed when comparing drowsiness ratings pre- and post-directed use period within the ECIG use group, collapsed across device power. For example, in the ECIG user group at 10 mg/ml, pre-directed mean drowsiness ratings were 26.5 (SEM=6.6) and significantly decreased to 17.1 [(SEM=4.4); $t(10)=2.7$, $p<0.05$]; at 15 mg/ml, mean drowsiness ratings were 38.1 (SEM=8.0) and significantly decreased to 19.8 [(SEM=4.7); $t(10)=3.5$, $p<0.05$].

No significant differences between or within groups were observed when comparing drowsiness ratings pre- and post-*ad lib* use period [*p*s>0.05], across liquid nicotine concentration [*p*s>0.025], or device power setting [*p*s>0.05].

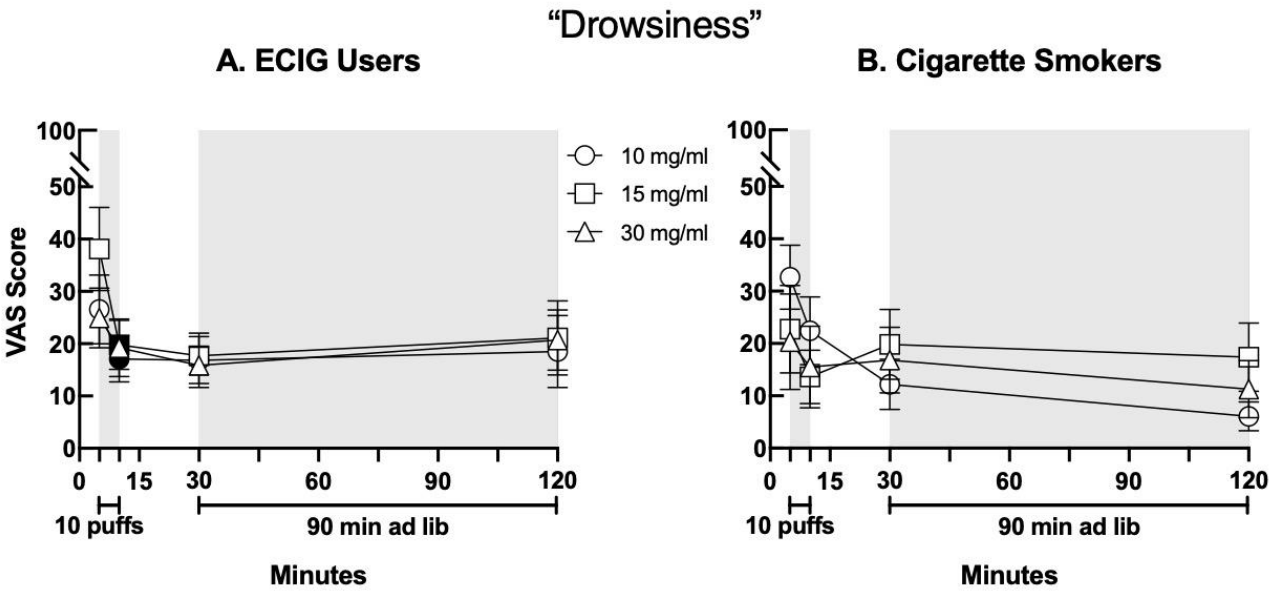

Figure 7. Mean data (± SEM) for the Hughes-Hatsukami "Drowsiness" item across time by liquid nicotine concentration and collapsed across device power setting (N=21).
ECIG users (n=11; Panel A) and cigarette smokers (n=10; Panel B) completed a directed, 10-puff use period and a 90-minute *ad libitum* use period. In all other respects, the figure is identical to Figure 2.

Hunger. For the item "hunger", significant interactions were observed for liquid nicotine concentration by time and device power by time (see Table 2); no significant main effect of group or significant interactions involving the group factor were observed. Therefore, "hunger" was re-analyzed using a three factor completely within-subject ANOVA (i.e., with no group factor), and significant interactions were observed for liquid nicotine concentration by time [$F(6,120)=2.3$, $p<0.05$] and device power by time [$F(3,60)=2.8$, $p<0.05$]. The means (± 1 SEM) for all conditions across time are depicted in Figure 8 Panels A (15 W) and B (30 W). As the figure shows, mean hunger ratings did not differ significantly by condition prior to the directed use period. However, mean hunger ratings decreased significantly over time for some conditions after the directed use period. Specifically, at 15 W for the 30 mg/ml condition, pre-directed mean hunger ratings were 28.6 (SEM=6.0) and significantly decreased to 17.7 [(SEM=3.9); $t(20)=2.2$, $p<0.05$]; at 30 W for the 15 mg/ml condition, mean hunger ratings were 43.7 (SEM=6.9) and significantly decreased to 20.6 [(SEM=4.1); $t(20)=3.3$, $p<0.05$]; at 30 W for the 30 mg/ml condition, mean hunger ratings were 44.3 (SEM=6.6) and significantly decreased to 22.4 [(SEM=5.0); $t(20)=4.2$, $p<0.05$].

Significant differences across device power setting were observed after the directed use period. Specifically, within the 15 mg/ml condition, mean hunger ratings were significantly greater 15 W condition (M=35.0, SEM=6.2) when compared to the 30 W condition [(M=20.6, SEM=4.1); $t(20)=2.3$, $p<0.05$].

Following the *ad lib* use period, mean hunger ratings were significantly different across time for some conditions. Specifically, at 15 W for the 30 mg/ml condition, pre-*ad lib* mean hunger ratings were 24.1 (SEM=4.6) and significantly increased to 38.1 [(SEM=7.2); $t(20)=-2.4$, $p<0.05$]; at 30 W for the 10 mg/ml condition, mean hunger ratings were 30.3 (SEM=5.4) and

significantly increased to 47.0 [(SEM=6.7); $t(20)=-3.2$, $p<0.05$]; at 30 W for the 15 mg/ml condition, mean hunger ratings were 29.7 (SEM=5.2) and significantly increased to 48.0 [(SEM=7.3; See Figure 8, Panel A); $t(20)=-2.6$, $p<0.05$].

No significant differences were observed after the *ad lib* use period across liquid nicotine concentration [*p*s>0.025] or device power setting [*p*s>0.05].

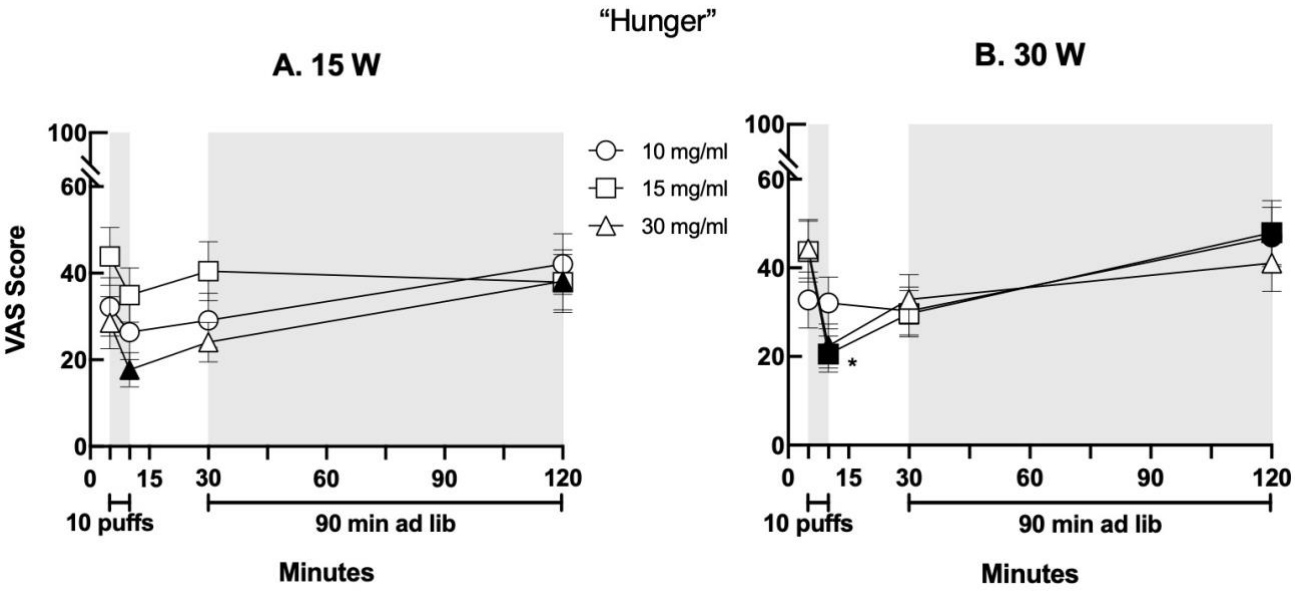

Figure 8. Mean data (± SEM) for the Hughes-Hatsukami "Hunger" item by condition and time (N=21).
In all other respects, the figure is identical to Figure 2.

Impatient. For the item "impatient", a significant main effect of time was observed (see Table 2); no significant main effect of group or significant interactions involving the group factor were observed. Therefore, "impatient" was re-analyzed using a three factor completely within-subject ANOVA (i.e., with no group factor), and a significant main effect of time was observed [$F(3,60)=12.6$, $p<0.05$]. The means (± 1 SEM) for all conditions across time are depicted in Figure 9 Panels A (15 W) and B (30 W). As the figure shows, mean impatient ratings did not differ by condition prior to the directed use period. However, impatient ratings decreased significantly over time for each liquid nicotine concentration level (i.e., 10, 15, 30 mg/ml) at each power setting (i.e., 15, 30 W) after the directed use period, [ts(20)>3.9, ps<0.05]. For example, at 15 W for the 10 mg/ml condition, pre-directed mean impatient ratings were 35.4 (SEM=6.6) and decreased to 17.5 (SEM=5.0; $p<0.05$); at 15 W for the 15 mg/ml condition, mean impatient ratings were 37.7 (SEM=6.8) and decreased to 14.7 (SEM=4.6; $p<0.05$); at 15 W for the 30 mg/ml condition, mean impatient ratings were 25.3 (SEM=6.3) and decreased to 13.4 (SEM=3.4; $p<0.05$). Similarly, at 30 W for the 10 mg/ml condition, pre-directed mean impatient ratings were 34.1 (SEM=7.2) and decreased to 17.5 (SEM=5.3; $p<0.05$); at 30 W for the 15 mg/ml condition, mean impatient ratings were 26.8 (SEM=5.9) and decreased to 7.3 (SEM=2.4; $p<0.05$); at 30 W for the 30 mg/ml condition, mean impatient ratings were 32.4 (SEM=6.5) and decreased to 9.9 (SEM=2.7; $p<0.05$).

Significant differences across device power setting were observed after the directed use period. Specifically, within the 15 mg/ml condition, mean impatient ratings were greater in the 15 W condition (M=14.7, SEM=4.6) when compared to the 30 W condition [(M=7.3, SEM=2.4); $t(20)=2.2$, $p<0.05$].

Following the *ad lib* use period, mean impatient ratings increased significantly over time in the 30 mg/ml;30 W condition only. Specifically, at 30 W for the 30 mg/ml condition, pre-*ad lib* mean impatient ratings were 8.3 (SEM=2.4) and increased significantly to 19.1 [(SEM=5.6; See Figure 9, Panel B); $t(20)=-2.4$, $p<0.05$].

No significant differences were observed after the *ad lib* use period across liquid nicotine concentration [*p*s>0.025] or device power setting [*p*s>0.05].

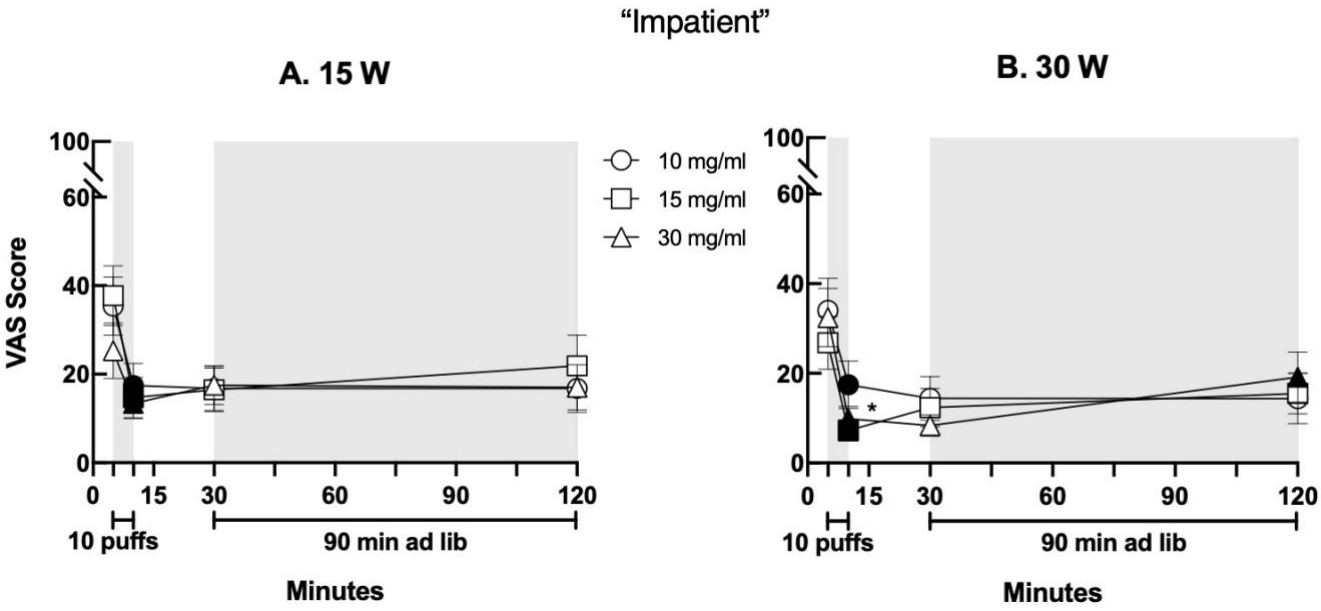

Figure 9. Mean data (± SEM) for the Hughes-Hatsukami "Impatient" item by condition and time (N=21).
In all other respects, the figure is identical to Figure 2.

Irritability. For the item "irritability/frustration/anger", a significant interaction was observed for time by group [$F(3,57)=4.8$, $p<0.05$; see Table 2]. The means (± 1 SEM) for both groups across time are depicted in Figure 10 Panels A (directed use period) and B (*ad lib* use period). As the figure shows, mean irritability ratings (collapsed across liquid nicotine concertation and device power) significantly differed by group prior to the directed use period. Specifically, mean irritability ratings were significantly greater in the cigarette smoker group (M=37.1, SEM=7.4) when compared to the ECIG user group (M=21.4, SEM=4.8) before the directed use period [$t(19)=-1.9$, $p<0.05$].

Significant differences were revealed when comparing irritability ratings pre- and post-directed use period within each group, collapsed across liquid nicotine concentration and power condition. For example, in the ECIG user group, pre-directed mean irritability ratings were 21.4 (SEM=4.8) and significantly decreased to 9.2 [(SEM=2.6); $t(10)=3.2$, $p<0.05$]; in the cigarette smoker group, mean irritability ratings were 37.1 (SEM=7.4) and significantly decreased to 11.0 [(SEM=2.4); $t(9)=4.0$, $p<0.05$].

Significant differences were revealed when comparing irritability ratings pre- and post-*ad lib* use period within the cigarette smoker group, collapsed across liquid nicotine concentration and power condition. Specifically, pre-*ad lib* mean irritability ratings were 11.7 (SEM=4.0) and significantly decreased to 5.2 [(SEM=3.1); $t(9)=3.4$, $p<0.05$].

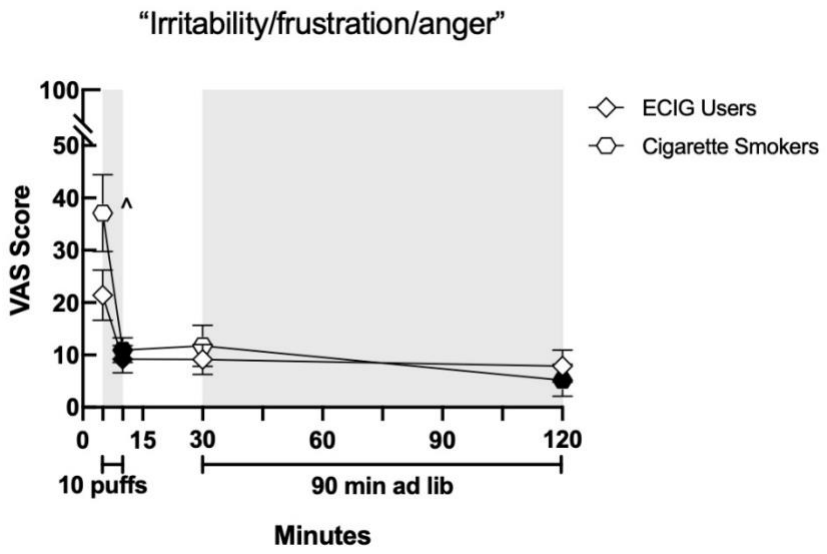

Figure 10. Mean data (± SEM) for the Hughes-Hatsukami "Irritability/frustration/anger" item by group, collapsed across liquid nicotine concentration and device power setting (N=21). ECIG users (n=11; diamond symbol) and cigarette smokers (n=10; hexagon symbol) completed a directed, 10-puff use period and a 90-minute *ad libitum* use period. In all other respects, the figure is identical to Figure 2.

Restless. For the item "restlessness", a significant main effect of time was observed (see Table 2); no significant main effect of group or significant interactions involving the group factor were observed. Therefore, "restlessness" was re-analyzed using a three factor completely within-subject ANOVA (i.e., with no group factor), and a significant main effect of time was observed [$F(3,60)=9.1$, $p<0.05$]. The means (± 1 SEM) for all conditions across time are depicted in Figure 11 Panels A (15 W) and B (30 W). As the figure shows, mean restlessness ratings did not differ significantly by condition prior to the directed use period. However, restlessness ratings decreased significantly over time for some conditions after the directed use period. For example, at 15 W for the 10 mg/ml condition, pre-directed mean restlessness ratings were 35.4 (SEM=7.4) and decreased to 14.5 [(SEM=3.4); $t(20)=3.8$, $p<0.05$]; at 15 W for the 15 mg/ml condition, mean restlessness ratings were 29.7 (SEM=5.6) and decreased to 15.0 [(SEM=4.7); $t(20)=2.9$, $p<0.05$]. Similarly, at 30 W for the 10 mg/ml condition, pre-directed mean restlessness ratings were 32.1 (SEM=6.9) and decreased to 18.5 [(SEM=4.8); $t(20)=2.2$, $p<0.05$]; at 30 W for the 30 mg/ml condition, mean restlessness ratings were 22.9 (SEM=5.1) and decreased to 11.1 [(SEM=2.7; See Figure 11, Panels A and B); $t(20)=2.6$, $p<0.05$].

No significant differences were observed after the directed use period across liquid nicotine concentration [ps>0.025] or device power setting [ps>0.05].

No significant differences were observed after the *ad lib* use period across time [ps>0.05] liquid nicotine concentration [ps>0.025] or device power setting [ps>0.05].

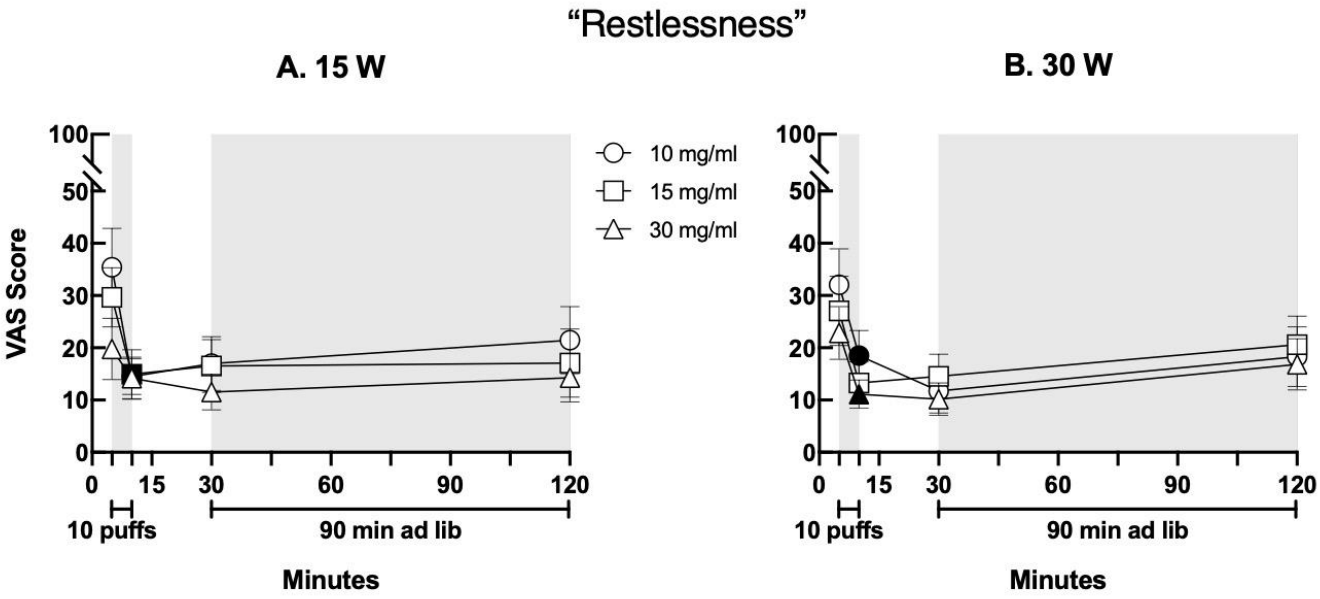

Figure 11. Mean data (± SEM) for the Hughes-Hatsukami "Restlessness" item by condition and time (N=21).
In all other respects, the figure is identical to Figure 2.

Urge. For the item "urges to vape/smoke", significant main effects of power and time were observed (see Table 2); no significant main effect of group or significant interactions involving the group factor were observed. Therefore, "urges to vape/smoke" was re-analyzed using a three factor completely within-subject ANOVA (i.e., with no group factor), and significant main effects of power [$F(1,20)=10.5$, $p<0.05$] and time [$F(3,60)=55.9$, $p<0.05$] were observed. The means (± 1 SEM) for all conditions across time are depicted in Figure 12 Panels A (15 W) and B (30 W). As the figure shows, mean urge ratings did not differ significantly by condition prior to the directed use period. However, urges to vape/smoke decreased significantly over time for each liquid nicotine concentration level (i.e., 10, 15, 30 mg/ml) at each power setting (i.e., 15, 30 W) after the directed use period, [ts(20)>5.7, ps<0.05]. For example, at 15 W for the 10 mg/ml condition, pre-directed mean urge ratings were 81.4 (SEM=4.5) and decreased to 54.8 (SEM=5.9; $p<0.05$); at 15 W for the 15 mg/ml condition, mean urge ratings were 81.2 (SEM=5.5) and decreased to 43.4 (SEM=7.3; $p<0.05$); at 15 W for the 30 mg/ml condition, mean urge ratings were 77.6 (SEM=6.4) and decreased to 42.1 (SEM=5.6; $p<0.05$). Similarly, at 30 W for the 10 mg/ml condition, pre-directed mean urge ratings were 73.4 (SEM=6.0) and decreased to 44.5 (SEM=5.5; $p<0.05$); at 30 W for the 15 mg/ml condition, mean urge ratings were 74.6 (SEM=6.0) and decreased to 38.0 (SEM=6.5; $p<0.05$); at 30 W for the 30 mg/ml condition, mean urge ratings were 77.6 (SEM=5.3) and decreased to 34.7 (SEM=5.1; $p<0.05$).

No significant differences were observed after the directed use period across liquid nicotine concentration [ps>0.025] or device power setting [ps>0.05].

Following the *ad lib* use period, mean urge ratings decreased significantly over time for each liquid nicotine concentration level at each power setting [ts(20)>10.7, ps<0.05]. For example, at 15 W for the 10 mg/ml condition, pre-*ad lib* mean urge ratings were 63.0 (SEM=5.9)

and decreased significantly to 35.2 (SEM=6.0; $p<0.05$); at 15 W for the 15 mg/ml condition, mean urge ratings were 57.6 (SEM=5.7) and decreased to 30.3 (SEM=6.5; $p<0.05$); at 15 W for the 30 mg/ml condition, mean urge ratings were 58.6 (SEM=4.9) and decreased to 26.7 (SEM=5.8; $p<0.05$; See Figure 12, Panel A). Similarly, at 30 W for the 10 mg/ml condition, pre-*ad lib* mean urge ratings were 58.2 (SEM=5.0) and decreased to 23.5 (SEM=5.1; $p<0.05$); at 30 W for the 15 mg/ml condition, mean urge ratings were 52.4 (SEM=5.0) and decreased to 22.4 (SEM=4.7; $p<0.05$); at 30 W for the 30 mg/ml condition, mean urge ratings were 46.6 (SEM=4.9) and decreased to 20.4 (SEM=4.1; $p<0.05$; See Figure 12, Panel B).

A significant difference across device power setting was observed after the *ad lib* use period. Specifically, within the 10 mg/ml condition, mean urge ratings were greater in the 15 W condition (M=35.2, SEM=6.0) when compared to the 30 W condition [(M=23.5, SEM=5.1); $t(20)=2.3$, $p<0.05$].

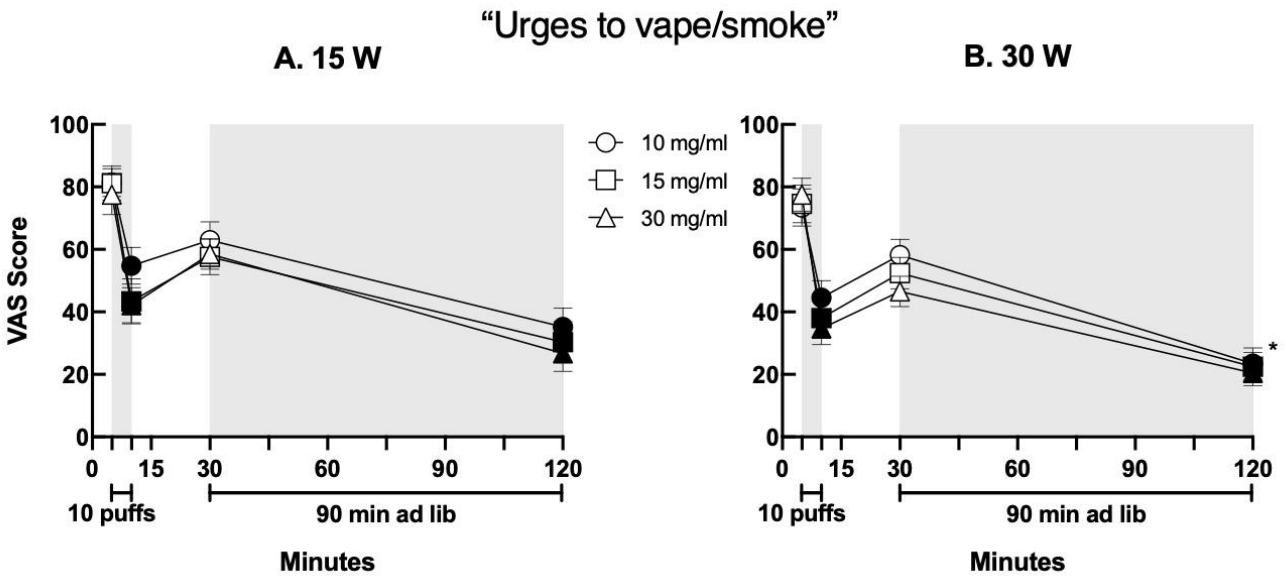

Figure 12. Mean data (± SEM) for the Hughes-Hatsukami "Urges to vape/smoke" item by condition and time (N=21).
In all other respects, the figure is identical to Figure 2.

Direct Effects of Nicotine

As displayed in Table 2, statistically significant interactions including the group factor were observed in items Confused, Heart Pounding, and Lightheaded (see Table 2) and these items were analyzed using a four factor mixed (within and between subjects) ANOVA. For the remaining DEN items, no significant interactions including the group factor were observed. Therefore, all remaining items were re-analyzed using a three factor completely within-subject ANOVA (i.e., with no group factor). Significant main effects of group and significant interactions including the group factor are detailed where appropriate.

Confused. For the item "confused", a significant three-way interaction was observed for power by time by group (see Table 2). The means (± 1 SEM) for both groups across time (collapsed across liquid nicotine concentration) are depicted in Figure 13 Panels A (directed use period) and B (*ad lib* use period). As the figure shows, confused means significantly differed by device power and group prior to the directed use period. Specifically, in the 15 W condition, pre-directed mean confused ratings were significantly greater in the ECIG user group (M=9.6, SEM=3.8) when compared to the cigarette smoker group [(M=2.1, SEM=1.6); $t(19)=1.8$, $p<0.05$].

No significant differences were revealed within groups across time [ps>0.05].

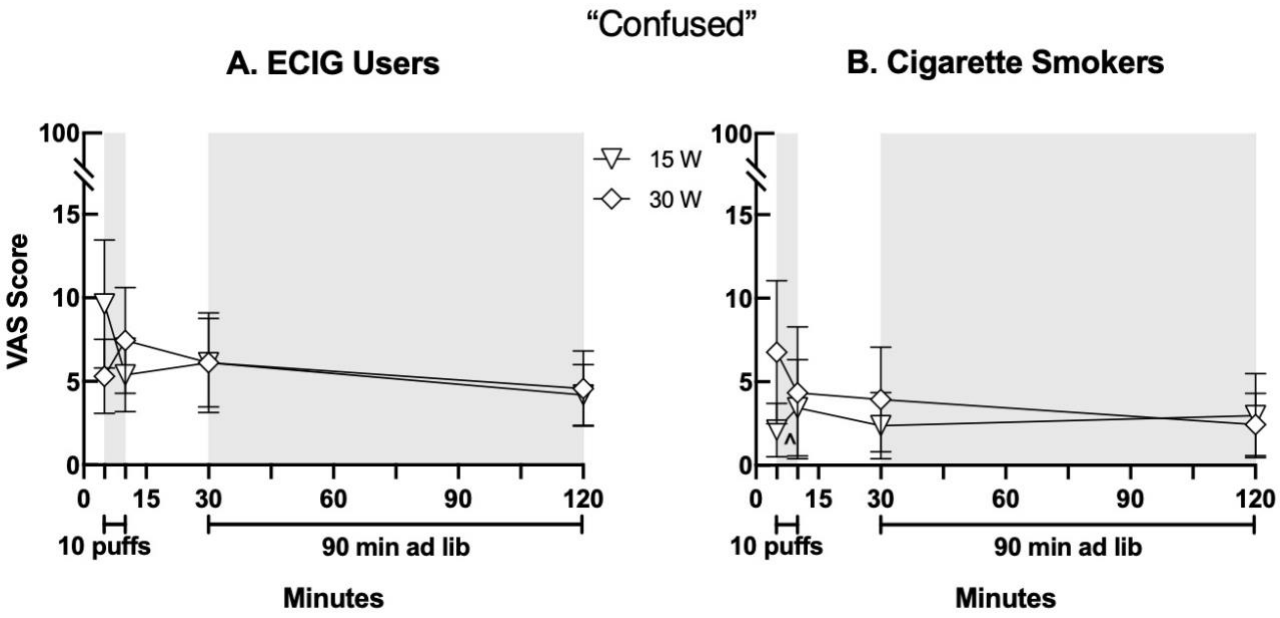

Figure 13. Mean data (± SEM) for the Direct Effects of Nicotine "Confused" item across time by device power setting and collapsed across liquid nicotine concentration (N=21).
ECIG users (n=11; Panel A) and cigarette smokers (n=10; Panel B) completed a directed, 10-puff use period and a 90-minute *ad libitum* use period based on liquid nicotine concentration condition (data are collapsed across this condition) and device power setting (15 W, inverted triangle symbol; 30 W, diamond symbol). Carat (^) indicates significant difference from ECIG user group at the same time point and condition. In all other respects, the figure is identical to Figure 2.

Dizzy. For the item "dizzy", a significant interaction was observed for device power by time (see Table 2); no significant main effect of group or significant interactions involving the group factor were observed. Therefore, "dizzy" was re-analyzed using a three factor completely within-subject ANOVA (i.e., with no group factor), and a significant interaction was observed for device power by time [$F(3,60)=5.4$, $p<0.05$]. The means (± 1 SEM) for all conditions across time are depicted in Figure 14 Panels A (15 W) and B (30 W). As the figure shows, mean dizzy ratings did not differ significantly by condition prior to the directed use period. However, mean dizzy ratings increased significantly in the higher-powered conditions after the directed use period. Specifically, within the 30 W condition, for the 10 mg/ml condition, pre-directed mean dizzy ratings were 4.0 (SEM=1.7) and increased significantly to 14.4 [(SEM=5.4); $t(20)=-2.6$, $p<0.05$]; for the 15 mg/ml condition, mean dizzy ratings were 5.3 (SEM=2.8) and increased significantly to 18.5 [(SEM=5.2); $t(20)=-2.6$, $p<0.05$]; for the 30 mg/ml condition, mean dizzy ratings were 7.1 (SEM=3.0) and increased significantly to 21.0 [(SEM=5.2; See Figure 14, Panel B); $t(20)=-3.1$, $p<0.05$].

No significant differences were observed after the directed use period across liquid nicotine concentration [ps>0.025] or device power setting [ps>0.05].

No significant differences were observed after the *ad lib* use period across time [ps>0.05] liquid nicotine concentration [ps>0.025] or device power setting [ps>0.05].

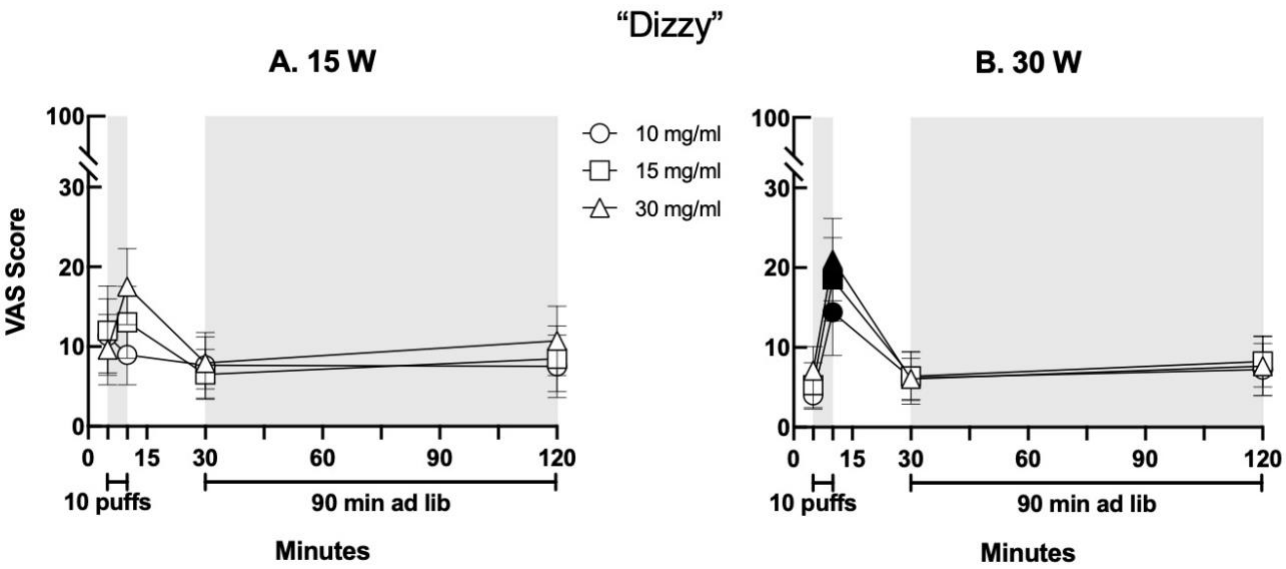

Figure 14. Mean data (± SEM) for the Direct Effects of Nicotine "Dizzy" item across conditions (N=21).

Participants completed a directed, 10-puff use period and a 90-minute *ad libitum* use period in six conditions based on liquid nicotine concentration condition (10 mg/ml, circle symbol; 15 mg/ml, square symbol; 30 mg/ml, triangle symbol) and device power setting (15 W, Panel A; 30 W Panel B). In all other respects, the figure is identical to Figure 2.

Heart Pounding. For the item "heart pounding", a significant four-way interaction was observed for nicotine by power by time by group and a significant three-way interaction was observed for nicotine by time by group (see Table 2). The means (± 1 SEM) by group and condition across time are depicted in Figure 15 Panels A (ECIG users) and B (cigarette smokers). Independent samples t-tests revealed a significant difference between groups following the directed use period [$p<0.05$]. Specifically, within the 30 mg/ml;30 W condition, mean heart pounding ratings were significantly greater in the ECIG user group (M=22.4, SEM=6.1) when compared to the cigarette smoker group [(M=6.4, SEM=4.4); $t(19)=2.1$, $p<0.05$].

Following the directed use period, mean heart pounding ratings increased significantly over time in the ECIG user group only. Specifically, in the 30 mg/ml;30 W condition, pre-directed mean heart pounding ratings were 3.5 (SEM=1.6) and increased significantly to 22.4 [(SEM=6.1); $t(10)=-3.4$, $p<0.05$]. Within the ECIG user group, significant differences across liquid nicotine concentration and device power setting were observed following the directed use period. Specifically, within the 30 W condition, mean heart pounding ratings were significantly greater in the 30 mg/ml condition (M=22.4, SEM=6.1) when compared to the 10 mg/ml condition [(M=7.9, SEM=3.0); $t(10)=-2.7$, $p<0.025$]. Additionally, in the 30 mg/ml condition, mean heart pounding ratings were significantly greater in the 30 W condition (M=22.4, SEM=6.1) when compared to the 15 W condition [(M=7.6, SEM=3.0); $t(10)=-2.6$, $p<0.05$].

Following the *ad lib* use period, a significant difference was observed when comparing across device power setting in the ECIG user group only. Specifically, within the 15 mg/ml condition, mean heart pounding ratings were significantly greater in the 30 W condition [(M=4.5, SEM=1.6) when compared to the 15 W condition [(M=1.7, SEM=1.2); $t(10)=-2.3$, $p<0.05$].

No significant differences were observed in the cigarette smoker group across time [*p*s>0.05] liquid nicotine concentration [*p*s>0.025] or device power setting [*p*s>0.05].

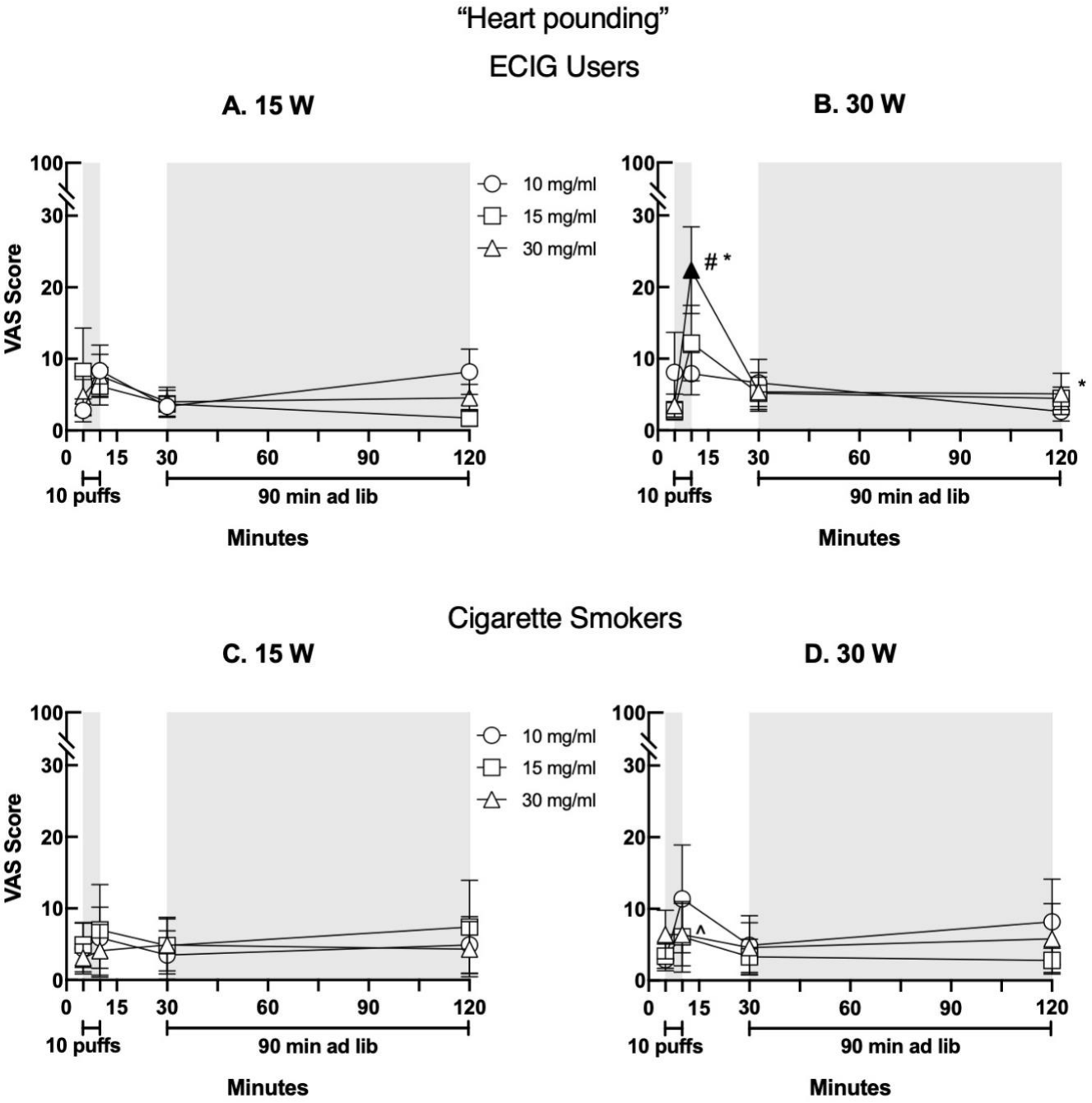

Figure 15. Mean data (± SEM) for the Direct Effects of Nicotine "Heart pounding" item across conditions (N=21).
ECIG users (n=11; Panels A & B) and cigarette smokers (n=10; Panels C & D) completed a directed, 10-puff use period and a 90-minute *ad libitum* use period based on liquid nicotine concentration condition (10 mg/ml, circle symbol; 15 mg/ml, square symbol; 30 mg/ml, triangle symbol) and device power setting (15 W, Panels A & C; 30 W, Panels B & D). In all other respects, the figure is identical to Figure 2.

Lightheaded. For the item "lightheaded", a significant three-way interaction was observed for power by time by group (see Table 2). The means (± 1 SEM) for both groups across time (collapsed across liquid nicotine concentration) are depicted in Figure 16 Panels A (directed use period) and B (*ad lib* use period). As the figure shows, mean lightheaded ratings differed by group and device power following the directed use period. However, independent samples t-tests did not reveal a significant difference between groups at this timepoint [*p*s>0.05].

When collapsed across liquid nicotine concentration conditions, significant differences across time and device power setting were revealed following the directed use period. For example, mean lightheaded ratings increased significantly over time following the directed use period in the ECIG user group. Specifically, in the 30 W condition, pre-directed mean lightheaded ratings were 8.4 (SEM=3.7) and increased significantly to 32.2 (SEM=7.3), [$t(10)=-3.8$, $p<0.05$]. Additionally, a significant difference across device power setting was observed following the directed use period in the ECIG user group. Specifically, mean lightheaded ratings were significantly lower in the 15 W condition (M=20.2, SEM=6.7) when compared to the 30 W condition [(M=32.2, SEM=7.3); $t(10)=-2.6$, $p<0.05$].

No significant differences in mean lightheaded ratings were observed following the directed use period in the cigarette smoker group when comparing across time or device power setting [*p*s>0.05].

No significant differences in mean lightheaded ratings were observed within groups following the *ad lib* use period [*p*s>0.05].

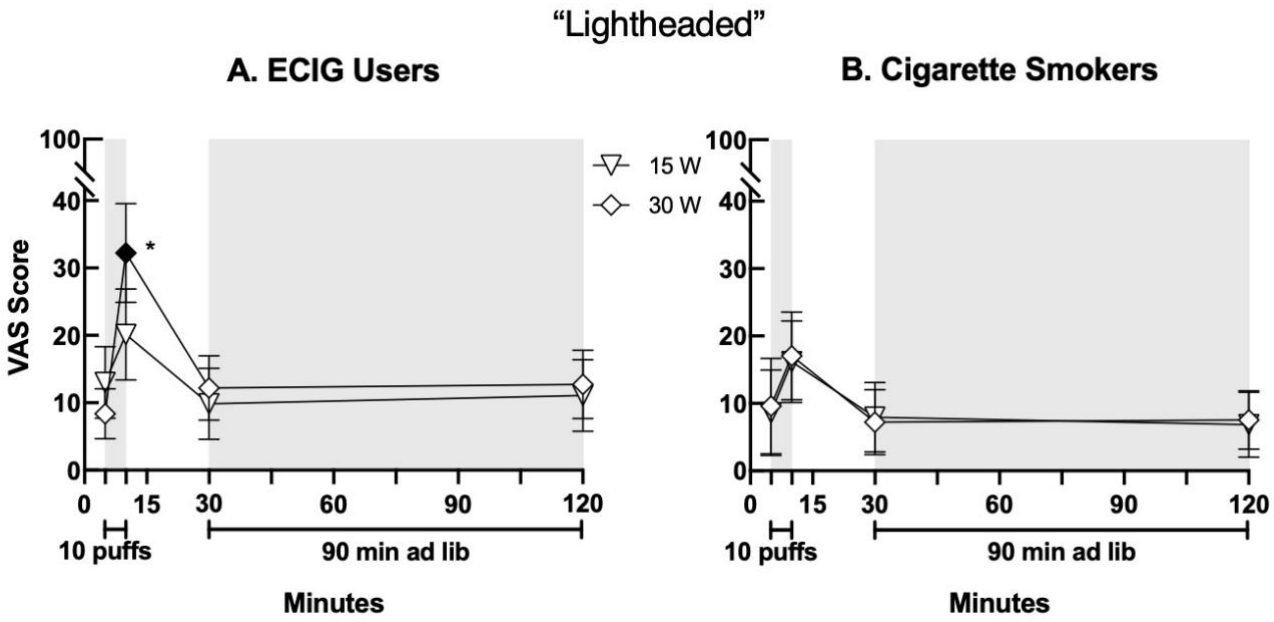

Figure 16. Mean data (± SEM) for the Direct Effects of Nicotine "Lightheaded" item across time by device power setting and collapsed across liquid nicotine concentration (N=21).
ECIG users (n=11; Panel A) and cigarette smokers (n=10; Panel B) completed a directed, 10-puff use period and a 90-minute *ad libitum* use period based on liquid nicotine concentration condition (data are collapsed across this condition) and device power setting (15 W, inverted triangle symbol; 30 W, diamond symbol). In all other respects, the figure is identical to Figure 2.

Nauseous. For the item "nauseous", a significant interaction was observed for device power by time (see Table 2); no significant main effect of group or significant interactions involving the group factor were observed. Therefore, "nauseous" was re-analyzed using a three factor completely within-subject ANOVA (i.e., with no group factor), and a significant interaction was observed for device power setting by time [$F(3,60)=4.6$, $p<0.05$]. The means (± 1 SEM) for all conditions across time are depicted in Figure 17 Panels A (15 W) and B (30 W). As the figure shows, mean nauseous ratings did not differ by condition at the pre-directed time point. However, mean nauseous ratings increased significantly over time in the 30 mg/ml;30 W condition following the directed use period. Specifically, at 30 W for the 30 mg/ml condition, pre-directed mean nauseous ratings were 2.8 (SEM=1.8) and increased significantly to 10.1 [(SEM=3.3; See Figure 17, Panel B); $t(20)=-2.5$, $p<0.05$].

A significant difference in mean nauseous ratings was observed following the directed use period when comparing across device power setting. Specifically, in the 30 mg/ml liquid concertation, mean nauseous ratings were greater in the 30 W condition (M=10.1, SEM=3.3) when compared to the 15 W condition [(M=4.1, SEM=1.9); $t(20)=-2.6$, $p<0.05$].

No significant differences were observed after the *ad lib* use period across time [*p*s>0.05], liquid nicotine concentration [*p*s>0.025], or device power setting [*p*s>0.05].

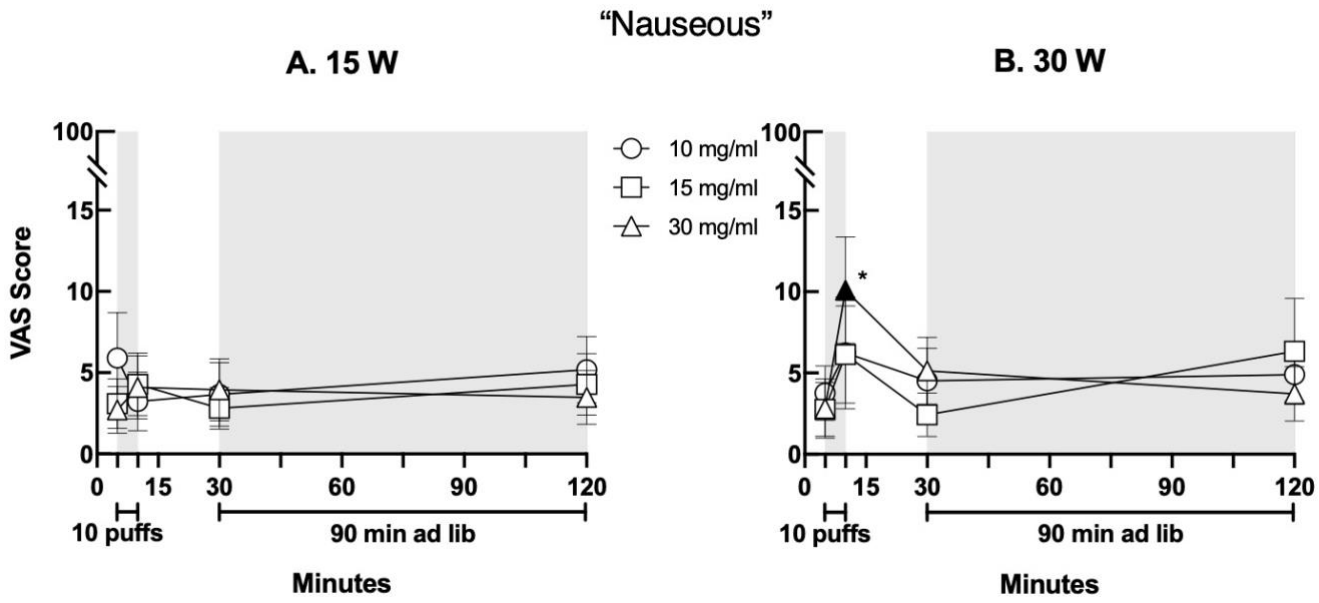

Figure 17. Mean data (± SEM) for the Direct Effects of Nicotine "Nauseous" item across conditions (N=21).
Participants completed a directed, 10-puff use period and a 90-minute *ad libitum* use period in six conditions based on liquid nicotine concentration condition (10 mg/ml, circle symbol; 15 mg/ml, square symbol; 30 mg/ml, triangle symbol) and device power setting (15 W, Panel A; 30 W Panel B). In all other respects, the figure is identical to Figure 2.

Nervous. For the item "nervous", a significant main effect of time was observed (see Table 2); no significant main effect of group or significant interactions involving the group factor were observed. Therefore, "nervous" was re-analyzed using a three factor completely within-subject ANOVA (i.e., with no group factor), and a significant main effect of time was observed [$F(3,60)=5.0$, $p<0.05$]. Post-hoc t-tests revealed no significant differences across time [ps>0.05], liquid nicotine concentration [ps>0.025], or device power setting [ps>0.05] following the directed use period or the *ad lib* use period.

Weak. For the item "weak", a significant three-way interaction was observed for liquid nicotine concentration by device power setting by time (see Table 2); no significant main effect of group or significant interactions involving the group factor were observed. Therefore, "weak" was re-analyzed using a three factor completely within-subject ANOVA (i.e., with no group factor), and a three-way interaction was observed for liquid nicotine concentration by device power setting by time [$F(6,120)=2.5$, $p=0.05$]. The means (± 1 SEM) for all conditions across time are depicted in Figure 18 Panels A (15 W) and B (30 W). As the figure shows, mean weak ratings did not decrease significantly over time after the directed use period [ps>0.05].

No significant differences in weak ratings were observed after the directed use period across liquid nicotine conditions [ps>0.025] or device power settings [ps>0.05].

Following the *ad lib* use period, mean weak ratings decreased significantly over time for the 30 mg/ml;30 W condition only. Specifically, at 30 W for the 30 mg/ml condition, pre-*ad lib*

use period mean weak ratings were 7.8 (SEM=2.5) and decreased significantly to 3.5 (SEM=1.5; See Figure 18, Panel B), [$t(20)=2.9$, $p<0.05$].

No significant differences were observed after the *ad lib* use period across liquid nicotine concentration [$ps>0.025$] or device power setting [$ps>0.05$].

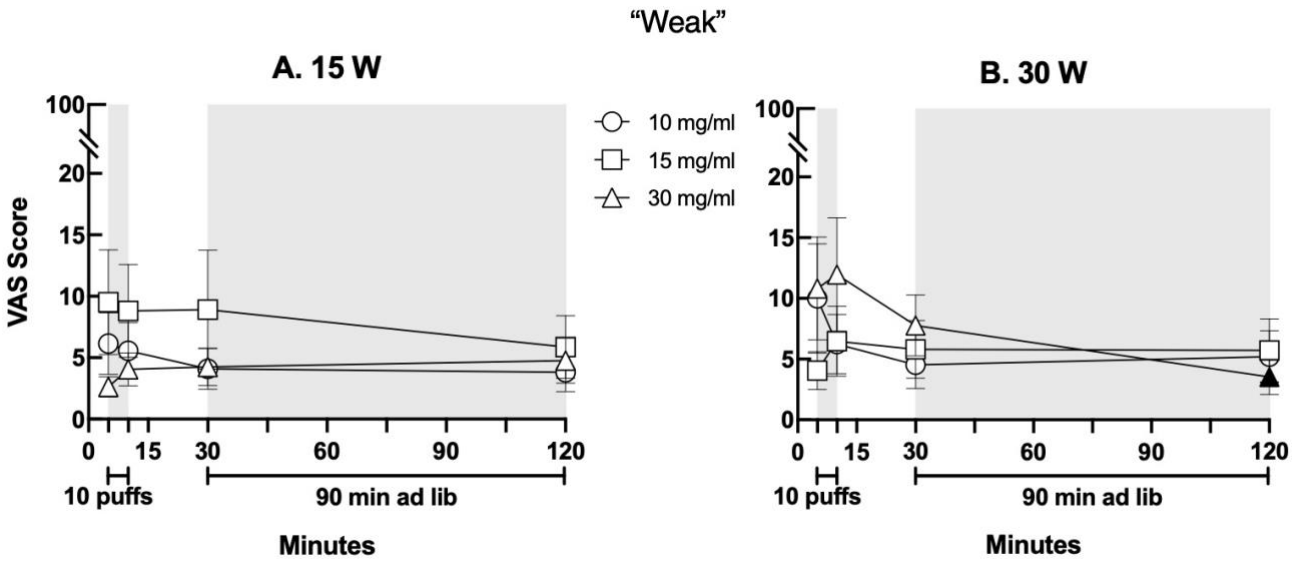

Figure 18. Mean data (± SEM) for the Direct Effects of Nicotine "Weak" item across conditions (N=21).

Participants completed a directed, 10-puff use period and a 90-minute *ad libitum* use period in six conditions based on liquid nicotine concentration condition (10 mg/ml, circle symbol; 15 mg/ml, square symbol; 30 mg/ml, triangle symbol) and device power setting (15 W, Panel A; 30 W Panel B). In all other respects, the figure is identical to Figure 2.

Direct Effects of ECIG Use

The direct effects of ECIG use was administered after ECIG use only. Therefore, the factor of time was not included in analysis and the directed and *ad lib* use periods were analyzed separately. The liquid concentration, power, and group factors are the same as all other measures. As displayed in Table 6, statistically significant interactions including the group factor were observed in items Right Now (directed use period only) and Sick (*ad lib* period only), and these items were analyzed using a three factor, mixed (within and between subjects) ANOVA. For the remaining items, no significant interactions including the group factor were observed. Therefore, all remaining items were re-analyzed using a two factor completely within-subject ANOVA (i.e., with no group factor). Significant main effects of group and significant interactions including the group factor are detailed where appropriate.

Table 6. Statistical Analysis Results for Three-Factor Mixed Analysis of Variance (Directed + *ad libitum* use periods).

	Nicotine		Power		Nicotine * Power		Nicotine * Group		Power * Group		Nicotine * Power * Group	
DE ECIG use	F	η_p^2	F	η_p^2	F	η_p^2	F	η_p^2	F	η_p^2	F	η_p^2
Awake												
Directed	0.4	.02	2.9	.13	0.1	.01	0.2	.01	0.1	.01	0.6	.03
Ad libitum	0.3	.02	1.9	.09	1.3	.06	1.2	.06	0.3	.02	0.2	.01
Calm												
Directed	0.2	.01	0.8	.04	0.3	.02	1.2	.06	0.0	.00	3.1	.14
Ad libitum	0.1	.00	0.5	.03	0.6	.03	0.2	.01	1.0	.05	0.1	.00
Concentrate												
Directed	0.2	.01	0.8	.04	0.1	.00	0.0	.00	0.2	.01	1.6	.08
Ad libitum	1.5	.08	0.3	.01	0.4	.02	0.6	.03	0.6	.03	0.4	.02
Dizzy												
Directed	1.8	.08	**16.1**	**.46**	0.5	.03	1.5	.07	**9.1**	**.32**	0.2	.01
Ad libitum	2.5	.12	2.1	.10	0.0	.00	1.7	.08	1.6	.08	0.8	.04
Pleasant												
Directed	**7.1**	**.27**	0.9	.05	0.3	.02	0.8	.04	0.9	.05	1.0	.05
Ad libitum	**3.8**	**.17**	0.0	.00	0.6	.03	0.1	.00	0.2	.01	0.2	.01
Reduce Hunger												
Directed	**4.1**	**.18**	0.1	.01	1.7	.08	0.1	.00	0.1	.01	1.0	.05
Ad libitum	**3.6**	**.16**	0.0	.00	1.3	.06	3.0	.14	2.5	.12	0.4	.02
Right Now												
Directed	**8.3**	**.30**	**35.6**	**.65**	1.1	.05	0.0	.00	**8.5**	**.31**	2.8	.13
Ad libitum	1.0	.05	**11.6**	**.38**	1.7	.08	1.8	.09	0.1	.01	1.5	.07
Satisfy												
Directed	1.5	.07	1.4	.07	1.1	.05	0.9	.05	0.0	.00	2.9	.13
Ad libitum	0.3	.02	0.6	.03	**4.2**	**.18**	0.7	.03	0.0	.00	1.8	.09
Sick												
Directed	2.5	.12	0.8	.04	0.8	.04	0.4	.02	1.5	.07	1.4	.07
Ad libitum	1.9	.09	0.3	.02	2.1	.10	1.8	.09	**5.3**	**.22**	0.8	.04
Taste Good												
Directed	**4.0**	**.18**	0.2	.01	0.0	.00	2.1	.10	0.0	.00	1.3	.06
Ad libitum	1.7	.08	**6.6**	**.26**	1.7	.08	0.0	.00	0.1	.01	0.9	.05

df N=(2,38); df P=(1,19); df N*P=(2,38); df N*G=(2,38); df P*G=(1,19); df N*P*G=(2,38)

Dizzy. For the item "did the e-cig make you dizzy?", a significant interaction for power by group [$F(1,19)=9.1$, $p<0.05$] and a main effect of power [$F(1,19)=16.1$, $p<0.05$; see Table 6] were observed following the directed use period. The means (+ 1 SEM) for each group (collapsed across liquid nicotine concentration) following the directed use period are depicted in Figure 19 Panels A (ECIG users) and B (cigarette smokers). As the figure shows, differences in mean "did the e-cig make you dizzy?" ratings based on device power setting were greater in the ECIG user group when compared to the cigarette smoker group. Independent samples t-tests revealed a significant difference between groups in the 30 mg/ml;30 W condition [$t(19)=2.6$, $p<0.05$] following the directed use period.

Following the directed use period, significant differences across device power setting were revealed within the ECIG user group. Specifically, within the 10 mg/ml condition, mean dizzy ratings were significantly greater in the 15 W condition (M=18.1, SEM=7.2) when compared to the 30 W condition [(M=34.3, SEM=9.2); $t(10)=-2.6$, $p<0.05$]; at 15 mg/ml, mean dizzy ratings were significantly greater in the 15 W condition (M=21.7, SEM=7.4) when compared to the 30 W condition [(M=41.3, SEM=10.9); $t(10)=-3.1$, $p<0.05$]; at 30 mg/ml, mean dizzy ratings were significantly greater in the 15 W condition (M=29.8, SEM=7.8) when compared to the 30 W condition [(M=58.0, SEM=9.3); $t(10)=-3.0$, $p<0.05$]. Additionally, a significant difference was revealed when comparing across liquid nicotine concentrations within the ECIG user group. Within the 30 W condition, mean dizzy ratings were significantly greater in the 10 mg/ml condition (M=34.3, SEM=9.2) when compared to the 30 mg/ml condition [(M=58.0, SEM=9.3); $t(10)=-2.9$, $p<0.025$] following the directed use period.

No significant differences were observed within the cigarette smoker group across liquid nicotine concentration [ps>0.025] or device power setting [ps>0.05].

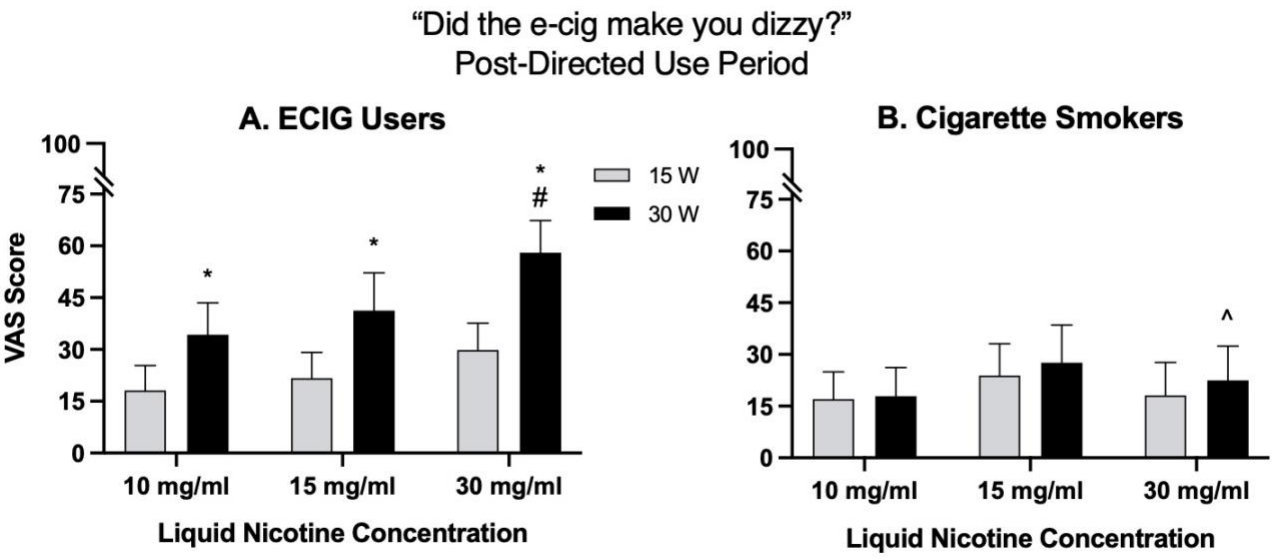

Figure 19. Mean data (+ SEM) for the Direct Effects of ECIG use "Did the e-cig make you dizzy?" item by liquid nicotine concentration and device power setting conditions (N=21). ECIG users (n=11; Panel A) and cigarette smokers (n=10; Panel B) completed a directed, 10-puff use period and a 90-minute *ad libitum* use period (not pictured) in six conditions based on liquid nicotine concentration condition (10 mg/ml, 15 mg/ml, 30 mg/ml) and device power setting (15 W, white bars; 30 W, black bars). Carat (^) indicates significant difference from ECIG user group at the same time point and condition. Number sign (#) indicates significant difference from 10 mg at same time point. Asterisks (*) indicate significant difference from 15 W at same time point.

Pleasant. For the item "was the e-cig pleasant?", a significant main effect of liquid nicotine concentration was observed following the directed use period and the *ad lib* use period (see Table 6); no significant main effect of group or significant interactions involving the group factor were observed. Therefore, "was the e-cig pleasant?" was re-analyzed using a two factor completely within-subject ANOVA (i.e., with no group factor), and a significant main effect of liquid nicotine concentration was observed following the directed use period [$F(2,40)=7.4$, $p<0.05$] and the *ad lib* use period [$F(2,40)=4.0$, $p<0.05$]. The means (+ 1 SEM) for all conditions are depicted in Figure 20 Panels A (post-directed use period) and B (post-*ad lib* use period). As the figure shows, "was the e-cig pleasant?" means decreased as liquid nicotine concentration increased following the directed period.

A significant difference across liquid nicotine concentration was observed after the directed use period. Specifically, within the 30 W condition, mean pleasant ratings were significantly greater in the 10 mg/ml condition (M=63.2, SEM=5.7) when compared to the 30 mg/ml condition [(M=43.5, SEM=6.2); $t(20)=2.6$, $p<0.025$].

No significant differences were observed after the *ad lib* use period across liquid nicotine concentration [$ps>0.025$] or device power setting [$ps>0.05$].

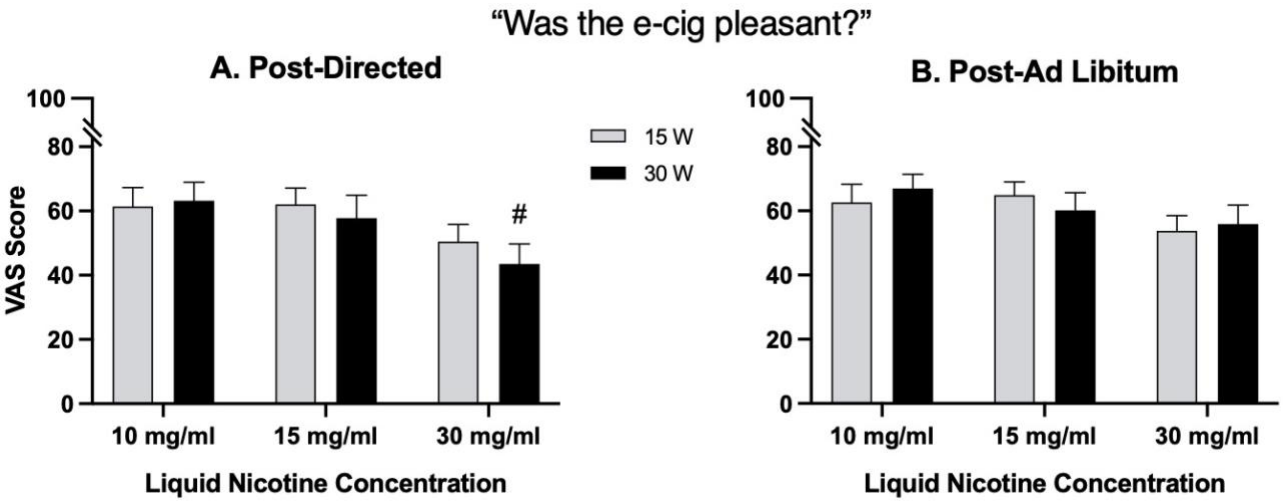

Figure 20. Mean data (+ SEM) for the Direct Effects of ECIG use "Was the e-cig pleasant?" item by liquid nicotine concentration and device power setting conditions (N=21).
Participants completed a directed, 10-puff use period (Panel A) and a 90-minute *ad libitum* use period (Panel B) in six conditions based on liquid nicotine concentration condition (10 mg/ml; 15 mg/ml; 30 mg/ml) and device power setting (15 W, gray bars; 30 W, black bars). Number sign (#) indicates significant difference from 10 mg at same time point. Asterisks (*) indicate significant difference from 15 W at same liquid nicotine concentration.

Reduce hunger. For the item "did the e-cig reduce your hunger for food?", a significant main effect of liquid nicotine concentration was observed following the directed use period and the *ad lib* use period (see Table 6); no significant main effect of group or significant interactions involving the group factor were observed. Therefore, "did the e-cig reduce your hunger for food?" was re-analyzed using a two factor completely within-subject ANOVA (i.e., with no group factor), and a significant main effect of liquid nicotine concentration was observed following the directed use period [$F(2,40)=4.4$, $p<0.05$] and the *ad lib* use period [$F(2,40)=3.6$, $p<0.05$]. The means (+ 1 SEM) for all conditions are depicted in Figure 21 Panels A (post-directed use period) and B (post-*ad lib* use period). As the figure shows, mean "did the e-cig reduce your hunger for food?" ratings differed by condition.

Following the directed use period, significant differences in mean reduced hunger ratings were observed across liquid nicotine conditions. Specifically, in the 15 W condition, mean reduced hunger ratings were significantly lower in the 10 mg/ml condition (M=15.1, SEM=4.2) when compared to the 15 mg/ml condition [(M=33.3, SEM=7.0); $t(20)=-2.6$, $p<0.025$] and to the 30 mg/ml condition [(M=32.1, SEM=7.1); $t(20)=-2.7$, $p<0.025$]. No significant differences in mean reduced hunger ratings were observed after the directed use period across device power settings [ps>0.05].

Following the *ad lib* use period, a significant difference across liquid nicotine concentration was observed. Specifically, in the 15 W conditions, mean reduced hunger ratings were significantly lower in the 10 mg/ml liquid nicotine condition (M=17.8, SEM=4.9) when compared to the 15 mg/ml condition [(M=28.3, SEM=6.1); $t(20)=-2.6$, $p<0.025$]. No significant differences were observed after the *ad lib* use period across device power setting [ps>0.05].

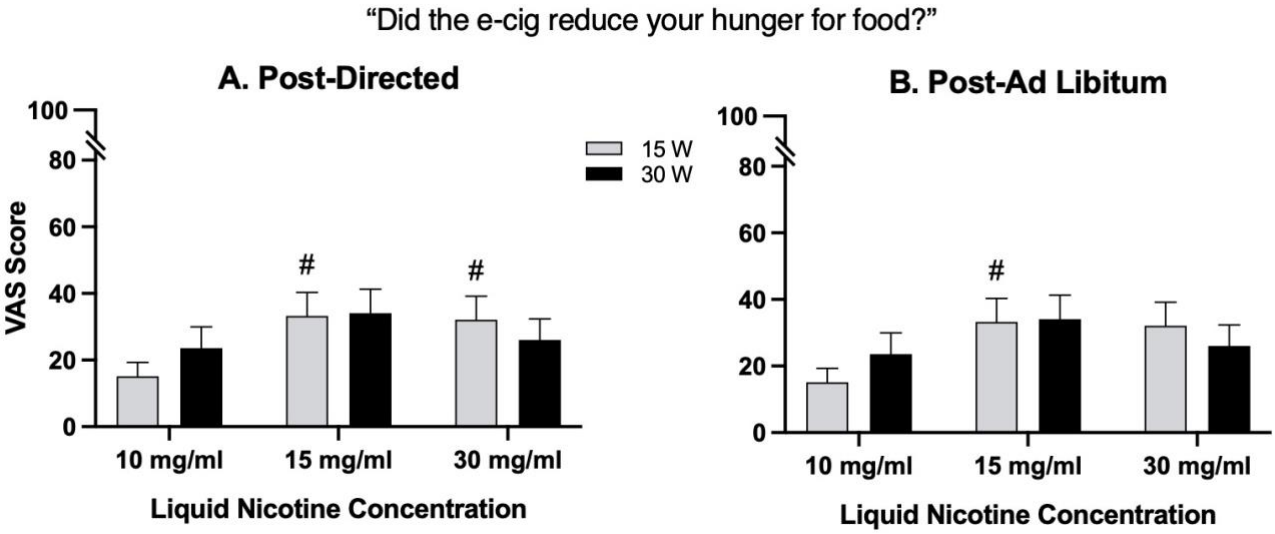

Figure 21. Mean data (+ SEM) for the Direct Effects of ECIG use "Did the e-cig reduce your hunger for food?" item by liquid nicotine concentration and device power setting conditions (N=21).
In all other respects, the figure is identical to Figure 20.

Right now. For the item "would you like to use another e-cig right now?", a significant interaction was observed for power by group following the directed use period (see Table 6). The means (+ 1 SEM) for each group (collapsed across liquid nicotine concentration) following the directed use period are depicted in Figure 22 Panels A (ECIG users) and B (cigarette smokers). As the figure shows, differences in mean "would you like to use another e-cig right now?" ratings based on device power setting were greater in the ECIG user group when compared to the cigarette smoker group. However, independent samples t-tests did not reveal a significant difference between groups [*p*s>0.05].

Significant differences across device power setting were revealed within groups following the directed use period. Specifically, in the ECIG user group, mean right now ratings were significantly greater in the 15 W condition (M=55.5, SEM=6.1) when compared to the 30 W condition [(M=35.8, SEM=6.6); $t(10)=5.6$, $p<0.05$]. Additionally, in the cigarette smoker group, mean right now ratings were significantly greater in the 15 W condition (M=49.3, SEM=7.5) when compared to the 30 W condition [(M=42.5, SEM=8.1); $t(9)=2.6$, $p<0.05$].

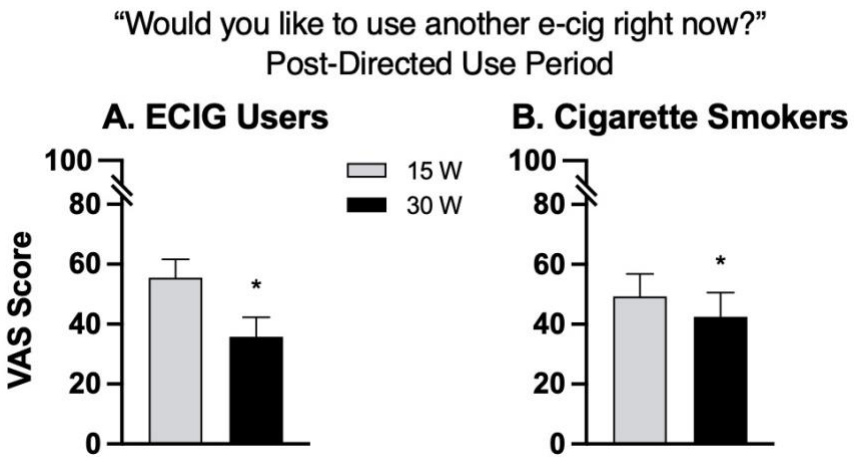

Figure 22. Mean data (+ SEM) for the Direct Effects of ECIG use "Would you like to use another e-cig right now?" item across conditions (N=21).
ECIG users (n=11; Panel A) and cigarette smokers (n=10; Panel B) completed a directed, 10-puff use period and a 90-minute *ad libitum* use period (not pictured) in six conditions based on liquid nicotine concentration condition (collapsed across this condition) and device power setting (15 W, white bars; 30 W, black bars). In all other respects, the figure is identical to Figure 19.

Satisfy. For the item "was the e-cig satisfying?", a significant interaction was observed for liquid nicotine concentration by power following the *ad lib* use period (see Table 6); no significant main effect of group or significant interactions involving the group factor were observed. Therefore, "was the e-cig satisfying?" was re-analyzed using a two factor completely within-subject ANOVA (i.e., with no group factor), and a significant interaction was observed for liquid nicotine concentration by power [$F(2,40)=3.3$, $p<0.05$] following the *ad lib* use period. The means (+ 1 SEM) for all conditions are depicted in Figure 23 Panels A (post-directed use period) and B (post-*ad lib* use period). No significant differences in mean "was the e-cig satisfying?" ratings were observed after the directed use period across liquid nicotine conditions [ps>0.025] or device power settings [ps>0.05].

A significant difference across device power setting was observed following the *ad lib* use period. Specifically, mean satisfy ratings were significantly different when comparing the 10 mg/ml liquid nicotine condition at 15 W (M=51.9, SEM=5.3) and 30 W [(M=65.0, SEM=5.3); $t(20)=-2.3$, $p<0.05$]. No significant differences were observed after the *ad lib* use period across liquid nicotine concentration [ps>0.025].

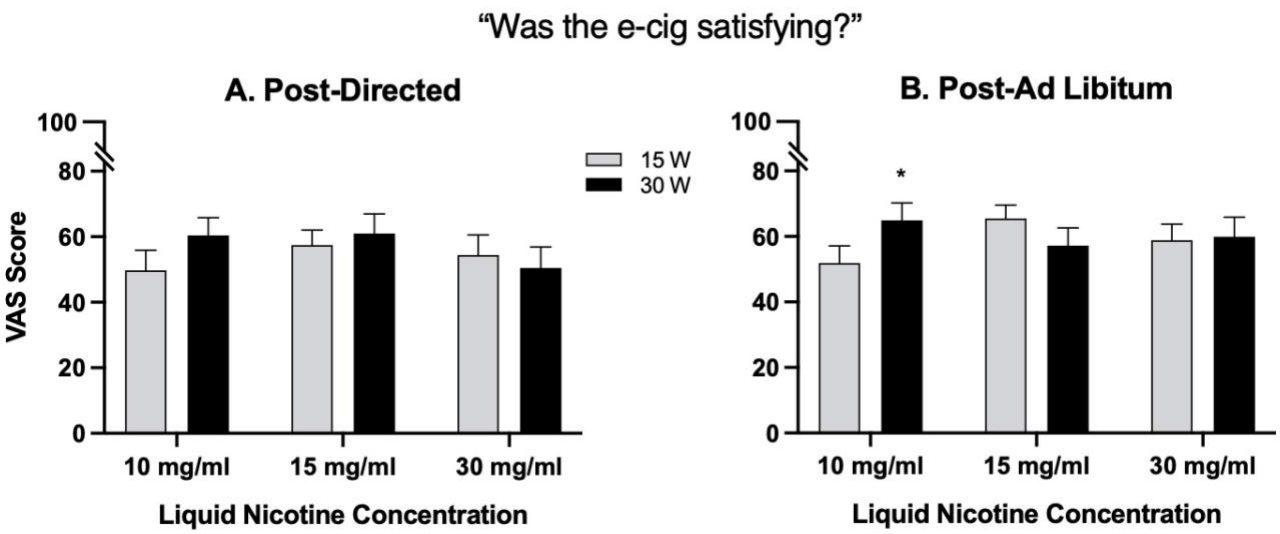

Figure 23. Mean data (+ SEM) for the Direct Effects of ECIG use "Was the e-cig satisfying?" item by liquid nicotine concentration and device power setting conditions (N=21).
In all other respects, the figure is identical to Figure 20.

Sick. For the item "did the e-cig make you sick?", a significant interaction was observed for power by group following the *ad lib* use period (see Table 6). However, independent samples t-tests did not reveal a significant difference between groups [*p*s>0.05]. Additionally, when collapsed across liquid nicotine concentration conditions, post-hoc tests did not reveal significant differences within groups [*p*s>0.05].

Taste good. For the item "did the e-cig taste good?", a significant main effect of liquid nicotine concentration was observed following the directed use period, as well as a significant main effect of power following the *ad lib* use period (see Table 6); no significant main effect of group or significant interactions involving the group factor were observed. Therefore, "did the e-cig taste good?" was re-analyzed using a two factor completely within-subject ANOVA (i.e., with no group factor), and a significant main effect of liquid nicotine concentration [$F(2,40)=3.6$, $p<0.05$] was observed following the directed use period, as well as a main effect of device power [$F(1,20)=6.8$, $p<0.05$] following the *ad lib* use period. The means (+ 1 SEM) for all conditions are depicted in Figure 24 Panels A (post-directed use period) and B (post-*ad lib* use period).

No significant differences in mean taste good ratings were observed after the directed use period across liquid nicotine concentration [*p*s>0.025] or device power setting [*p*s>0.05].

A significant difference across device power setting was observed following the *ad lib* use period. Specifically, mean taste good ratings were significantly different when comparing the 10 mg/ml liquid nicotine condition at 15 W (M=58.8, SEM=5.8) and 30 W [(M=71.5, SEM=3.8); $t(20)=-2.1$, $p<0.05$]. No significant differences were observed after the *ad lib* use period across liquid nicotine concentration [*p*s>0.025].

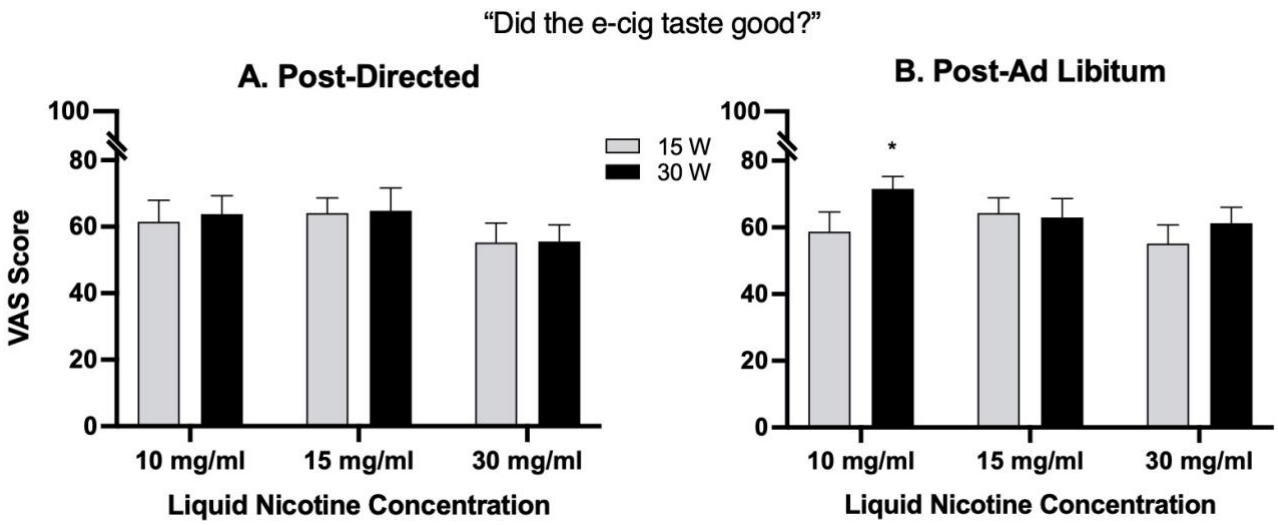

Figure 24. Mean data (+ SEM) for the Direct Effects of ECIG use "Did the e-cig taste good?" item by liquid nicotine concentration and device power setting conditions (N=21).
In all other respects, the figure is identical to Figure 20.

General Labeled Magnitude Score

The general labeled magnitude score (gLMS) was administered after ECIG use only. Therefore, the factor of time was not included in analysis and the directed and *ad lib* use periods were analyzed separately. The liquid concentration, power, and group factors are the same as all other measures. No statistically significant interactions including the group factor were observed (see Table 7). Therefore, all items were re-analyzed using a two factor completely within-subject ANOVA (i.e., with no group factor or time factor), and these results are reported here (see Table 8).

Table 7. Statistical Analysis Results for Three-Factor Mixed Analysis of Variance (Directed + *ad libitum* use periods).

	Nicotine		**Power**		**Nicotine * Power**		**Nicotine * Group**		**Power * Group**		**Nicotine * Power * Group**	
gLMS	F	η_p^2	F	η_p^2	F	η_p^2	F	η_p^2	F	η_p^2	F	η_p^2
Flavor												
Directed	0.7	.04	**17.2**	**.48**	1.1	.05	0.4	.02	1.3	.06	0.2	.01
Ad libitum	1.6	.08	**12.9**	**.40**	0.8	.04	0.6	.03	3.3	.15	1.3	.06
Harshness												
Directed	**20.7**	**.52**	3.5	.16	0.4	.02	0.9	.05	0.7	.04	0.2	.01
Ad libitum	**8.3**	**.30**	1.8	.09	1.4	.07	0.1	.00	0.1	.01	0.2	.01
Throat Hit												
Directed	**21.3**	**.53**	**14.8**	**.44**	1.3	.07	0.5	.03	1.1	.06	0.3	.01
Ad libitum	**8.2**	**.30**	**8.7**	**.32**	2.3	.11	0.1	.01	0.5	.03	0.5	.03

df N=(2,38); df P=(1,19); df N*P=(2,38); df N*G=(2,38); df P*G=(1,19); df N*P*G=(2,38)

Table 8. Statistical Analysis Results for Two-Factor Mixed Analysis of Variance (Directed + *ad libitum* use periods).

	Nicotine		**Power**		**Nicotine * Power**	
gLMS	F	η_p^2	F	η_p^2	F	η_p^2
Flavor						
Directed	.71	.03	**16.5**	**.45**	1.1	.05
Ad libitum	1.5	.07	**11.0**	**.36**	.81	.04
Harshness						
Directed	**21.3**	**.52**	3.4	.15	0.38	.02
Ad libitum	**8.6**	**.30**	1.9	.09	1.5	.07
Throat Hit						
Directed	**22.1**	**.53**	**14.4**	**.42**	1.3	.06
Ad libitum	**8.7**	**.30**	**8.8**	**.31**	2.2	.10

df N=(2,40); df P=(1,20); df N*P=(2,40)

Flavor. For ratings of flavor, a significant main effect of power was observed following the directed use period and the *ad lib* period (see Table 8). The means (+ 1 SEM) for all conditions are depicted in Figure 25 Panels A (post-directed use period) and B (post-*ad lib* use period). As the figure shows, significant differences across device power setting were observed after the directed and *ad lib* use periods.

Following the directed use period, within the 15 mg/ml condition, mean flavor ratings were significantly greater in the 30 W condition (M=37.7, SEM=5.3) when compared to the 15 W condition [(M=26.7, SEM=3.4); $t(20)=-2.5$, $p<0.05$]. Additionally, in the 30 mg/ml condition, mean flavor ratings were significantly greater in the 30 W condition (M=37.6, SEM=5.1) when compared to the 15 W condition [(M=28.4, SEM=6.0); $t(20)=-2.5$, $p<0.05$].

Following the *ad lib* use period, within the 15 mg/ml condition, mean flavor ratings were significantly greater in the 30 W condition (M=35.7, SEM=5.3) when compared to the 15 W condition [(M=24.1, SEM=3.6); $t(20)=-2.9$, $p<0.05$]. No significant differences were observed across liquid nicotine concentration [ps>0.025].

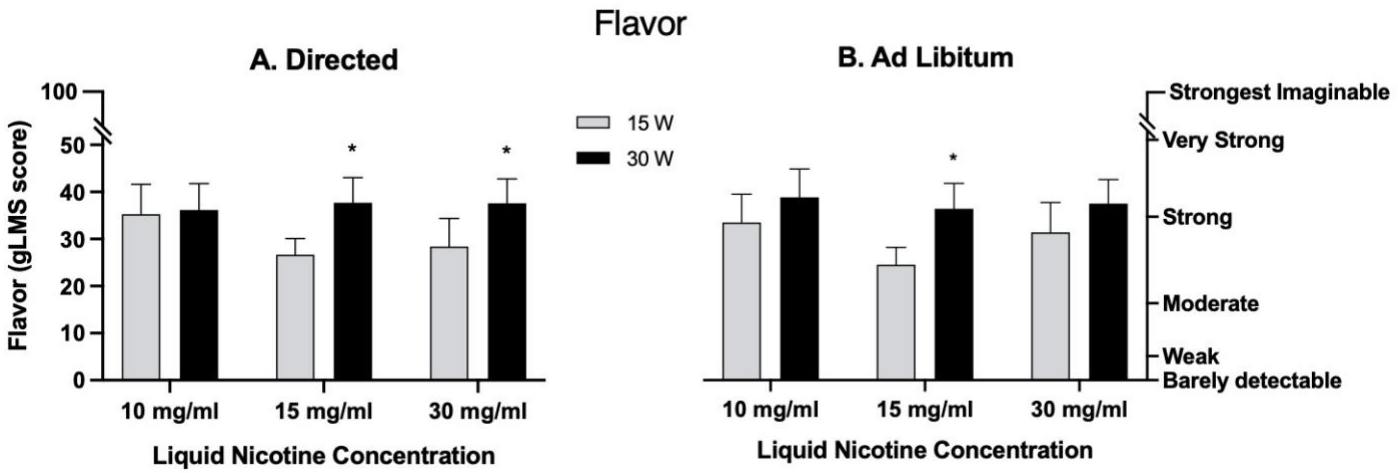

Figure 25. Mean data (+ SEM) for the General Labeled Magnitude Scale "Flavor sensation" item by liquid nicotine concentration and device power setting conditions (N=21).
Participants completed a directed, 10-puff use period (Panel A) and a 90-minute *ad libitum* use period (Panel B) in six conditions based on liquid nicotine concentration condition (10 mg/ml; 15 mg/ml; 30 mg/ml) and device power setting (15 W, white bars; 30 W, black bars). Number sign (#) indicates significant difference from 10 mg at same time point. Asterisks (*) indicate significant difference from 15 W at same liquid nicotine concentration.

Harshness. For ratings of harshness, a significant main effect of liquid nicotine concentration was observed following the directed use period and the *ad lib* use period (see Table 8). The means (+ 1 SEM) for all conditions are depicted in Figure 26 Panels A (post-directed use period) and B (post-*ad lib* use period). As the figure shows, harshness ratings increased as liquid nicotine concentration and device power setting increased.

Following the directed use period, in the 10 mg/ml condition, mean harshness ratings were significantly greater in the 30 W condition (M=25.2, SEM=5.3) when compared to the 15 W condition [(M=14.6, SEM=3.0); $t(20)=-3.2$, $p<0.05$]. Significant differences across liquid nicotine concentration were also observed after the directed use period. Specifically, at 15 W, mean harshness ratings were greater in the 30 mg/ml condition (M=31.4, SEM=5.2) when compared to the 10 mg/ml condition [(M=14.6, SEM=3.0); $t(20)=-3.8$, $p<0.025$]. Additionally, at 30 W, mean harshness ratings were significantly lower in the 10 mg/ml condition (M=25.2, SEM=5.3) when compared to the 15 mg/ml condition [(M=31.8, SEM=5.7); $t(20)=-2.8$, $p<0.025$] and to the 30 mg/ml condition [(M=41.7, SEM=6.4); $t(20)=-4.9$, $p<0.025$].

Following the *ad lib* use period, significant differences across device power setting and liquid nicotine concentration were observed. Within the 10 mg/ml condition, mean harshness ratings were significantly greater in the 30 W condition (M=27.7, SEM=5.6) when compared to the 15 W condition [(M=16.4, SEM=4.1); $t(20)=-2.7$, $p<0.05$]. Comparing across liquid nicotine concentration, at 15 W, mean harshness ratings were significantly greater in the 30 mg/ml condition (M=32.4, SEM=6.0) when compared to the 10 mg/ml condition [(M=16.4, SEM=4.1); $t(20)=-2.9$, $p<0.025$]. Similarly, at 30 W, mean harshness ratings were significantly greater in the

30 mg/ml condition (M=38.0, SEM=5.9) when compared to the 10 mg/ml condition [(M=27.7, SEM=5.6); $t(20)=-3.8$, $p<0.025$].

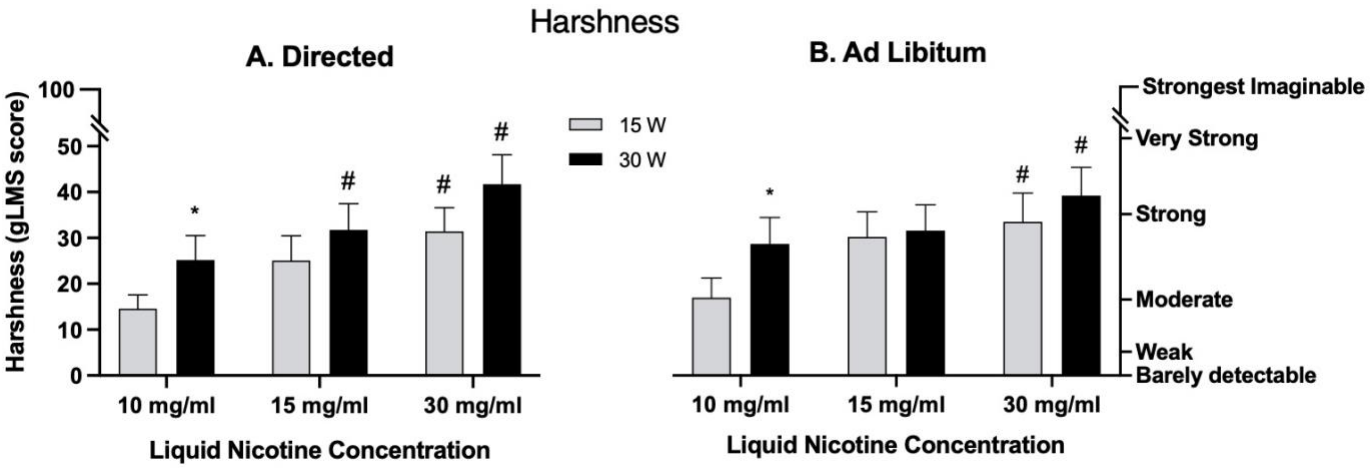

Figure 26. Mean data (+ SEM) for the General Labeled Magnitude Scale "Harshness" item by liquid nicotine concentration and device power setting conditions (N=21).
In all other respects, the figure is identical to Figure 25.

Throat Hit. For ratings of throat hit, a significant main effect of nicotine concentration was observed following the directed use period and the *ad lib* use period (see Table 8). Additionally, a significant main effect of power was observed following the directed use period and the *ad lib* use period (see Table 8). The means (+ 1 SEM) for all conditions are depicted in Figure 27 Panels A (post-directed use period) and B (post-*ad lib* use period). As the figure shows, throat hit ratings increased as liquid nicotine concentration and device power setting increased following ECIG use.

Following the directed use period, within the 10 mg/ml liquid nicotine concentration condition, mean throat hit ratings were significantly greater in the 30 W condition (M=32.1, SEM=5.3) when compared to the 15 W condition (M=14.3, SEM=3.1) after the directed use period, [$t(20)=-4.7$, $p<0.05$]. Additionally, within the 30 mg/ml liquid nicotine concentration condition, mean throat hit ratings were significantly greater in the 30 W condition (M=47.0, SEM=5.3) when compared to the 15 W condition [(M=34.8, SEM=5.2); $t(20)=-3.2$, $p<0.05$]. Significant differences across liquid nicotine concentration were also observed after the directed use period. Specifically, within the 15 W condition, mean throat hit ratings were lower in the 10 mg/ml condition (M=14.3, SEM=3.1) when compared to the 15 mg/ml condition [(M=27.4, SEM=5.3); $t(20)=-4.0$, $p<0.05$] and to the 30 mg/ml condition [(M=34.8, SEM=5.2); $t(20)=-4.7$, $p<0.05$]. Additionally, within the 30 W condition, mean throat hit ratings were significantly greater in the 30 mg/ml condition (M=47.0, SEM=5.3) when compared to the 10 mg/ml condition [(M=32.1, SEM=5.3); $t(20)=-5.2$, $p<0.025$].

Following the *ad lib* use period, significant differences across device power setting and liquid nicotine concentration were observed. Similar to the pattern observed following the directed use period, throat hit ratings increased as liquid nicotine concentration and device power

setting increased following the *ad lib* user period. Within the 10 mg/ml condition, mean throat hit ratings were significantly greater in the 30 W condition (M=32.3, SEM=5.6) when compared to the 15 W condition [(M=19.5, SEM=4.4); $t(20)=-3.6$, $p<0.05$]. Additionally, within the 30 mg/ml condition, mean throat hit ratings were significantly greater in the 30 W condition (M=41.6, SEM=5.4) when compared to the 15 W condition [(M=32.5, SEM=6.0); $t(20)=-2.8$, $p<0.05$]. Comparing across liquid nicotine concentration, within the 15 W condition, mean throat hit ratings were greater in the 30 mg/ml condition (M=32.5, SEM=6.0) when compared to the 10 mg/ml condition [(M=19.5, SEM=4.4); $t(20)=-3.0$, $p<0.025$]. Similarly, within the 30 W condition, mean throat hit ratings were significantly greater in the 30 mg/ml condition (M=41.6, SEM=5.4) when compared to the 10 mg/ml condition [(M=32.3, SEM=5.6); $t(20)=-4.2$, $p<0.025$].

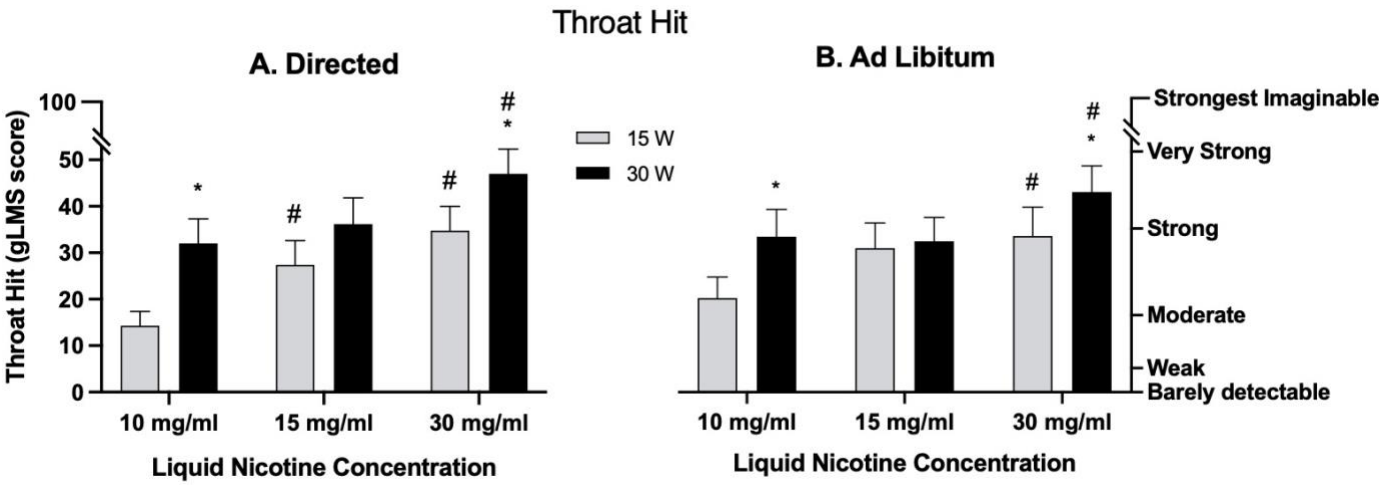

Figure 27. Mean data (+ SEM) for the General Labeled Magnitude Scale "Throat hit" item by liquid nicotine concentration and device power setting conditions (N=21).
In all other respects, the figure is identical to Figure 25.

Discussion

Overview

This study examined the interaction of protonated nicotine liquid concentration and device power output on physiological measures (plasma nicotine concentration, heart rate), user behavior (puff topography) and subjective effects. Previous research has established that freebase liquid nicotine concentration and device power output influence plasma nicotine concentration and other outcome measures related to ECIG use. However, this study is the first to manipulate nicotine concentration and device power systematically when the ECIG liquid contains a majority of protonated nicotine. Findings from the present study suggest that liquid nicotine concentration and device power influence ECIG nicotine delivery, user behavior, and subjective effects associated with use of ECIG devices containing protonated nicotine. For example, increases in one or both of these factors lead to increases in plasma nicotine concentration. This effect, also seen with liquids that are primarily freebase nicotine (Hiler et al., 2020), has implications regarding the consequences that might be expected in response to ECIG regulations that attempt to control nicotine delivery by limiting one factor (e.g., liquid protonated nicotine concentration) when other factors (e.g., device power and/or puff duration) are unregulated. Results from this study support the combination of regulations aimed at limiting the rate at which nicotine is emitted from the ECIG (nicotine flux; Shihadeh & Eissenberg, 2015) and regulations aimed at limiting puff duration. By limiting nicotine flux and puff duration simultaneously, regulators may gain control over the nicotine delivery of ECIGs.

Physiological Effects

In this study, physiological effects involved measurement of participant plasma nicotine concentration and heart rate. Overall, plasma nicotine concentration and HR significantly

increased in all conditions after ECIG use, indicating that all combinations of liquid nicotine concentration and device power reliably delivered physiologically active nicotine doses.

With respect to how liquid nicotine concentration influenced plasma nicotine, following the directed use period (i.e., 10 puffs, 30 sec IPI), within the 15 W conditions, the mean increase in plasma nicotine concentration was more pronounced (but not significantly so) in the 30 mg/ml nicotine condition (M=14.0, SD=12.7) when compared to the 10 mg/ml nicotine condition (M=9.1, SD=7.7). This observation that higher liquid nicotine concentration is associated with greater plasma nicotine concentration is consistent with previous studies of freebase nicotine ECIG liquids. Specifically, increases in liquid nicotine concentration were associated with significantly greater increases in plasma nicotine concentration (Hiler et al., 2017, 2020; Dawkins et al., 2016). Examining the effect of device power setting (independent of liquid nicotine concentration), within the 10 mg/ml nicotine condition, the observed mean increase in plasma nicotine concentration was more pronounced (but not significantly so) in the 30 W condition (M=11.7, SD=10.0) when compared to the 15 W condition (M=9.1, SD=7.7). This observation that greater device power output is associated with higher plasma nicotine concentration is consistent with previous studies of ECIGs. Specifically, increases in device power output were associated with significantly greater increases in plasma nicotine concentration (Hiler et al., 2020; Wagener et al., 2017). Looking at the combined effects of liquid nicotine concentration and device power output, in the condition with highest liquid nicotine concentration and higher device power setting (30 mg/ml;30 W), the mean (SD) increase in plasma nicotine concentration following 10 puffs was 19.1 ng/ml (19.2), the highest nicotine plasma concentration observed following the directed use period (see Figure 2; p. 53). Additionally, the mean increase in plasma nicotine concentration observed in the 30 mg/ml;30 W

condition was significantly greater when compared to the increase observed in the 10 mg/ml;15 W condition [(M=9.1, SD=7.7); $t(20)=-3.0$, $p<0.05$]. In sum, these observations that plasma nicotine concentration increased following 10 puffs demonstrate that liquid nicotine concentration and device power setting alone and in combination influence the nicotine delivery of ECIGs containing protonated nicotine, making attending to both of these factors essential to any regulatory action(s) aimed at limiting the nicotine delivery of ECIGs.

Similarly, HR significantly increased in all conditions during ECIG use, confirming the physiological effects of the observed increases in plasma nicotine. With respect to how liquid nicotine concentration influenced HR, following the directed use period (i.e., 10 puffs, 30 sec IPI), within the 15 W conditions, the mean increase in HR was more pronounced (but not significantly so) in the 30 mg/ml nicotine condition (M=13.6, SD=8.0) when compared to the 10 mg/ml nicotine condition (M=11.1, SD=6.3). This observation that higher liquid nicotine concentration is associated with increased HR is consistent with a previous study of freebase nicotine ECIG liquids. Specifically, increases in liquid nicotine concentration were associated with significantly greater increases in HR (Hiler et al., 2017). Examining the effect of device power setting (independent of liquid nicotine concentration), within the 10 mg/ml nicotine condition, the observed mean increase in HR was more pronounced (but not significantly so) in the 30 W condition (M=13.2, SD=9.3) when compared to the 15 W condition (M=11.1, SD=6.3). This observation that greater device power output is associated with increased HR is consistent with a previous study of ECIGs. Specifically, increases in device power output were associated with significantly greater increases in HR (Hiler et al., 2020). Looking at the combined effects of liquid nicotine concentration and device power output, in the condition with highest liquid nicotine concentration and higher device power setting (30 mg/ml;30 W), the mean (SD)

increase in HR following 10 puffs was 19.3 bpm (8.7), the highest HR observed following the directed use period (see Figure 3; p. 56). Additionally, the mean increase in HR observed in the 30 mg/ml;30 W condition was significantly greater when compared to the HR increase observed in the 10 mg/ml;15 W condition [(M=11.1, SD=6.3); $t(20)=-6.2$, $p<0.05$]. In sum, these observations that HR increased following 10 puffs demonstrate that liquid nicotine concentration and device power setting alone and in combination influence the nicotine delivery and, correspondingly, the cardiovascular response of ECIGs containing protonated nicotine.

Significant differences in plasma nicotine concentration following ECIG use were not detected between liquid nicotine concentration or device power output following the *ad lib* use period, suggesting that the effects of these two factors on nicotine delivery may be influenced by user behavior.

Puff Topography

In this study, puff topography involved measurement of puff count and duration, as well as inter-puff interval (IPI) and average flow rate. Significant differences were observed in puff duration and IPI (see Tables 3-5, p. 58-59), indicating that liquid nicotine concentration and device power influenced puff topography during ECIG use.

Significant differences in puff duration during the directed use period based on liquid nicotine concentration and device power setting were observed. With respect to how liquid nicotine concentration influenced puff duration, during the directed use period (i.e., 10 puffs, 30 sec IPI), within the 15 W conditions, mean puff duration was significantly shorter in the 30 mg/ml nicotine condition (M=2.9, SD=0.2) when compared to the 10 mg/ml nicotine condition (M=3.7, SD=0.3). This observation that higher liquid nicotine concentration is associated with shorter puff duration is consistent with previous studies of freebase nicotine ECIG liquids

(Dawkins et al., 2016, 2018; Hiler et al., 2017). Examining the effect of device power setting (independent of liquid nicotine concentration), within the 10 mg/ml nicotine condition, the observed mean puff duration was significantly shorter in the 30 W condition (M=2.5, SD=0.2) when compared to the 15 W condition (M=3.7, SD=0.3). This observation that greater device power output is associated with shorter puff duration is consistent with previous studies of ECIGs (Farsalinos et al., 2018; Hiler et al., 2020). Looking at the combined effects of liquid nicotine concentration and device power output, in the condition with highest liquid nicotine concentration and higher device power setting (30 mg/ml;30 W), the mean (SD) puff duration was 2.0 sec (0.2), the shortest puff duration observed during the directed use period (see Table 4; p. 59). Additionally, the mean puff duration observed in the 30 mg/ml;30 W condition was significantly shorter when compared to the puff duration observed in the 10 mg/ml;30 W condition (M=2.5, SD=0.2) and the 30 mg/ml;15 W condition (M=2.9, SD=0.2; see *Results* section).

Similarly, changes in IPI during the *ad lib* use period based on liquid nicotine concentration and device power setting were observed. With respect to how liquid nicotine concentration influenced IPI, during the *ad lib* use period, within the 15 W conditions, mean IPI was longer (but not significantly so) in the 30 mg/ml nicotine condition (M=187.0, SD=23.6) when compared to the 15 mg/ml nicotine condition (M=126.8, SD=13.2), and the 10 mg/ml nicotine condition (M=184.7, SD=47.3). This observation that higher liquid nicotine concentration is associated with longer IPI is consistent with previous ECIG research using freebase nicotine ECIG liquids. Specifically, increases in liquid nicotine concentration were associated with significantly longer IPI (Dawkins et al., 2018). Examining the effect of device power setting (independent of liquid nicotine concentration), within the 30 mg/ml nicotine

condition, the observed mean IPI was significantly longer in the 30 W condition (M=298.0, SD=65.0) when compared to the 15 W condition (M=187.0, SD=23.6). This observation that greater device power output is associated with longer IPI is consistent with previous studies of ECIGs (Kimber et al., 2021, see suppl. Figure S1). Looking at the combined effects of liquid nicotine concentration and device power output, in the condition with highest liquid nicotine concentration and higher device power setting (30 mg/ml;30 W), the mean (SD) IPI was 298.0 sec (65.0), the longest IPI observed during the *ad lib* use period (see Table 4; p. 59). Additionally, the mean IPI observed in the 30 mg/ml;30 W condition was significantly longer when compared to the IPI observed in the 10 mg/ml;15 W condition [(M=184.7, SD=47.3); $t(18)=2.4$, $p<0.05$].

Significant differences in puff count during the *ad lib* use period were observed within the ECIG user group only (see *Group Differences*, below). Nonetheless, non-significant changes in puff count based on liquid nicotine concentration and device power setting were observed. With respect to how liquid nicotine concentration influenced puff count, during the *ad lib* use period, within the 15 W conditions, mean puff count was lower (but not significantly so) in the 30 mg/ml nicotine condition (M=35.4, SD=7.2) when compared to the 10 mg/ml nicotine condition (M=50.1, SD=12.9). This observation that higher liquid nicotine concentration is associated with fewer puffs is consistent with previous studies of freebase nicotine ECIG liquids (Dawkins et al., 2016, 2018; Hiler et al., 2020). Examining the effect of device power setting (independent of liquid nicotine concentration), within the 10 mg/ml nicotine condition, the observed mean puff count was lower (but not significantly so) in the 30 W condition (M=44.1, SD=8.3) when compared to the 15 W condition (M=50.1, SD=12.9). This observation that greater device power output is associated with fewer puffs is consistent with previous studies of

ECIGs (Farsalinos et al., 2018). Looking at the combined effects of liquid nicotine concentration and device power output, in the condition with highest liquid nicotine concentration and higher device power setting (30 mg/ml;30 W), the mean (SD) puff count was 34.6 (11.0), the fewest puffs observed during the *ad lib* use period (see Table 4; p. 59).

In sum, these observations of changes in puff topography during ECIG use demonstrate that liquid nicotine concentration and device power setting alone and in combination influence puffing behavior during use of ECIGs containing protonated nicotine. Additionally, the observed differences in puff topography may be influenced by direct effects of nicotine and/or other sensations associated with ECIG use (e.g., harshness), suggesting that the effects of liquid concentration and/or device power on puff topography may be moderated by subjective effects.

Subjective Effects

In this study, subjective effects involved measurement of abstinence symptom severity, the direct effects of nicotine, and the direct effects of ECIG use. Additionally, this study measured specific sensations associated with ECIG product use (via the gLMS). Overall, significant differences were observed on measures of abstinence symptom severity, the direct effects of nicotine (see Table 2), and the direct effects of ECIG use (see Table 6). Also, significant differences were observed in all gLMS items (see Tables 7 and 8). These differences indicate that liquid nicotine concentration and device power influenced the subjective profile of ECIGs containing protonated nicotine.

Following the directed use period, all abstinence symptoms (i.e., Hughes-Hatsukami items) were reduced, with significant reductions observed on some VAS items. Significant reductions in abstinence symptom ratings following the directed use period (i.e., 10 puffs, 30 sec IPI) were observed in all conditions for the items assessing “Craving,” “Impatient,” and “Urges

to vape/smoke." With respect to how liquid nicotine concentration influenced craving ratings, following the directed use period (i.e., 10 puffs, 30 sec IPI), within the 15 W conditions, the mean reduction in craving ratings was more pronounced (but not significantly so) in the 30 mg/ml nicotine condition (M=32.5, SD=32.8) when compared to the 10 mg/ml nicotine condition (M=25.7, SD=25.6). This observation that higher liquid nicotine concentration is associated with more pronounced reduction in craving ratings is consistent with previous studies of freebase nicotine ECIG liquids (Dawkins et al., 2018). Specifically, increases in liquid nicotine concentration were associated with significantly more pronounced reduction in craving ratings (Hiler et al., 2017, 2020). Examining the effect of device power setting (independent of liquid nicotine concentration), within the 10 mg/ml nicotine condition, the observed mean reduction in craving ratings was more pronounced (but not significantly so) in the 30 W condition (M=30.9, SD=26.8) when compared to the 15 W condition (M=25.7, SD=25.6). This observation that greater device power output is associated with more pronounced reduction in craving ratings is consistent with previous studies of ECIGs. Specifically, increases in device power output were associated with significantly more pronounced reduction in craving ratings (Hiler et al., 2020). Looking at the combined effects of liquid nicotine concentration and device power output, in the condition with highest liquid nicotine concentration and higher device power setting (30 mg/ml;30 W), the mean (SD) reduction in craving ratings following 10 puffs was 51.6 (27.5), the greatest reduction in craving ratings observed following the directed use period (see Figure 5; p. 72). Additionally, the mean reduction in craving ratings observed in the 30 mg/ml;30 W condition was significantly more pronounced when compared to the reduction observed in the 10 mg/ml;15 W condition [(M=25.7, SD=25.6); $t(20)=-3.5$, $p<0.05$]. Similar effects were observed after the directed use period for items "Impatient" and "Urges to

vape/smoke." In sum, these observations that ratings of abstinence symptoms were reduced following 10 puffs demonstrate that liquid nicotine concentration and device power setting alone and in combination influence the abstinence symptom suppression of ECIGs containing protonated nicotine.

Significant differences were observed for items that measured the direct effects of nicotine and the direct effects of ECIG use. Significant increases in ratings of the direct effects of nicotine were observed following the directed use period (i.e., 10 puffs, 30 sec IPI) for the items "Dizzy" and "Nauseous." Examining the effect of device power setting (independent of liquid nicotine concentration), significant increases in mean "Dizzy" ratings following the directed use period were observed within the 30 W conditions only. Looking at the combined effects of liquid nicotine concentration and device power output, in the condition with highest liquid nicotine concentration and higher device power setting, a significant increase in nauseous ratings following the directed use period was observed in the 30 mg/ml;30 W condition only. These observations that higher liquid nicotine concentration and increased device power output are associated with increased ratings of the direct effects of nicotine are consistent with previous studies of ECIGs. Specifically, increases in one or both of these factors were associated with significant increases in ratings of the direct effects of nicotine (Hiler et al., 2017, 2020; Dawkins et al., 2018).

Similar effects based on liquid nicotine concentration and/or device power output were observed for the direct effects of ECIG use items "Did the e-cig make you dizzy?" (within the ECIG user group only; see *Group Differences*) and "Did the e-cig reduce your hunger for food?" following the directed use period. Interestingly, ratings of "Was the e-cig satisfying?" were significantly greater in the 10 mg/ml;30 W condition (M=65.0, SEM=5.3) when compared to the

10 mg/ml;15 W condition (M=51.9, SEM=5.3) following the *ad lib* use period. A similar effect was observed for the item "Did the e-cig taste good?", suggesting that device power output influenced ECIG palatability at lower liquid nicotine concentrations. In sum, these observations that increases in the direct effects of nicotine and the direct effects of ECIG use ratings increased as liquid nicotine concentration and device power output increased demonstrate that both of these factors, alone and in combination, influence the direct effects of ECIGs containing protonated nicotine.

Significant differences in specific sensations associated with ECIG use (i.e., flavor sensation, harshness, and throat hit) based on liquid nicotine concentration and device power setting were observed. For example, significant differences in mean ratings of "Harshness" based on liquid nicotine concentration and device power setting condition were observed following both ECIG use periods. With respect to how liquid nicotine concentration influenced craving ratings, following the *ad lib* use period, within the 15 W conditions, mean harshness ratings were significantly greater in the 30 mg/ml nicotine condition (M=32.4, SEM=6.0) when compared to the 10 mg/ml nicotine condition (M=16.4, SEM=4.1). Examining the effect of device power setting (independent of liquid nicotine concentration), within the 10 mg/ml nicotine condition, observed mean harshness ratings were significantly greater in the 30 W condition (M=27.7, SEM=5.6) when compared to the 15 W condition (M=16.4, SEM=4.1). Looking at the combined effects of liquid nicotine concentration and device power output, in the condition with highest liquid nicotine concentration and higher device power setting (30 mg/ml;30 W), mean (SD) harshness ratings were 38.0 (5.9), the highest ratings of harshness observed following the *ad lib* period (see Figure 26; p. 124). Additionally, mean harshness ratings exceeded the "Strong" label in the 30 mg/ml;30 W condition only. These observations that higher liquid nicotine

concentration and greater device power output are associated with greater sensation ratings are consistent with previous studies of ECIGs (Dawkins et al., 2016; Hiler et al., 2020). Similar effects were observed in gLMS items “flavor sensation” and “throat hit.” In sum, these observations of increased ratings of sensations associated with ECIG use as liquid nicotine concentration and device power setting were increased demonstrate that both of these factors, alone and in combination, influence the specific sensations of ECIGs containing protonated nicotine.

Furthermore, the observed differences in subjective effects may be an influence on puff topography. Specifically, higher liquid concentration and greater device power output was associated with increased ratings of “Dizzy,” “Nauseous,” and “Harshness.” These results suggest that liquid concentration and device power influence user experience, with increased subjective ratings as these factors are increased. Significant decreases in puff duration followed a similar pattern, with observations of decreased puff duration as liquid concentration and device power were increased. As users experience more pronounced direct effects and sensations associated with ECIG use, a decrease in puff duration may be adopted in response to sensation changes (i.e., harshness) and/or in an effort to titrate nicotine dose.

Considered together, these observations of changes in abstinence symptom severity, the direct effects of nicotine, the direct effects of ECIG use, and specific sensations associated with ECIG use demonstrate that liquid nicotine concentration and device power setting alone and in combination influence the subjective profile of ECIGs containing protonated nicotine. Additionally, the influence of these factors on subjective profile may moderate the changes observed in puff topography. Specifically, puffing behavior may be adjusted in response to

changes in specific sensations associated with ECIG use (due to increased harshness) and/or in response to the direct effects of nicotine/ECIG use (in an effort to titrate dose).

Group Differences

Participants were separated into two distinct groups based on experience with ECIGs (ECIG users, N=11; ECIG naïve cigarette smokers, N=10), and differences between these groups were observed for some measures. Overall, significant group differences were observed in puff topography (e.g., puff duration) and subjective measures (e.g., "heart pounding" and "did the e-cig make you dizzy?"), indicating that experience with ECIGs may influence puffing behavior and subjective effects. Specifically, ECIG users took significantly longer puffs than cigarette smokers in all conditions during the *ad lib* use period. This effect is consistent with research on ECIGs containing freebase nicotine (Farsalinos et al., 2015; Hiler et al., 2017). Also, ECIG users took fewer puffs (though not significantly so) than cigarette smokers, possibly providing an explanation for the lack of group differences in plasma nicotine concentration. Additionally, significant differences between groups were observed pre-directed use period for some subjective items (i.e., difficulty concentrating, irritability, lightheaded), suggesting differences in abstinence symptom severity and thus, potentially, dependence level (i.e., greater abstinence symptom severity at baseline may reflect more dependence). However, groups did not significantly differ on measures of nicotine dependence (see Table 1, p. 46). Significant group differences were observed following the directed use period for some items measuring the direct effects of nicotine and ECIG use (i.e., heart pounding and "did the e-cig make you dizzy?"). Specifically, mean ratings of heart pounding and dizziness were significantly greater in the ECIG users compared to the cigarette smokers following the directed use period (i.e.,10 puffs). This effect is consistent with research on ECIGs containing freebase nicotine (Hiler et al., 2017).

These observations indicate that experience with ECIGs is associated with changes in puffing behavior, as well as changes in the subjective profile of ECIGs. Importantly, significant group differences were not observed in plasma nicotine concentration or HR following ECIG use. Unfortunately, the present study was not powered adequately to examine group differences, and further examination of the effect of experience with ECIGs on the physiological and subjective effects of ECIGs containing protonated nicotine is warranted, including dependence level as assessed by abstinence symptom severity during periods where no nicotine self-administration is permitted.

Limitations

There are several limitations in the present study. First, this study was not designed to examine differences between groups based on experience with ECIGs, gender, or flavor preference. Differences between groups based on ECIG experience were detected, but this study may have lacked sensitivity to characterize fully differences based on participants' prior ECIG experience. Additionally, this study may have lacked sensitivity to detect differences based on participant demographics or flavor preference. Future studies would benefit from larger sample sizes in order to detect potential differences based on participant characteristics. Additionally, examinations of flavor preference in future studies may benefit from the inclusion of an unflavored liquid as a placebo control.

Second, the use of a single variable wattage ECIG device (Kanger Sub Box Mini) in this study may not be indicative of the typical device used by experienced ECIG users. In fact, the most popular ECIG device type used in the US is the "pod mod." However, characteristics of ECIG devices vary widely (e.g., battery voltage, liquid composition, wick material), and the majority of "pod mod" ECIGs do not allow the power of the device to be changed/manipulated.

The use of one variable wattage device was necessary to minimize potential threats to internal validity, ensuring that device characteristics (besides those being examined, e.g., power setting) were unlikely to influence study results. Future studies would benefit from examining the influence of additional device characteristics (e.g., liquid composition, wick material; see Karam et al., 2021; Talih et al., 2020) on the nicotine delivery and subjective profile of ECIGs.

Third, the absence of a freebase nicotine condition does not allow a direct comparison to protonated nicotine at different liquid nicotine concentrations and device power settings. This limitation is important considering the subjective outcomes (e.g., harshness), as protonated nicotine is often characterized as a less harsh alternative to freebase nicotine, especially at higher concentrations (Duell et al., 2020; Henningfield et al., 2004; Pankow, 2001). However, past research on ECIGs has been conducted primarily using freebase nicotine, as the vast majority of ECIG liquids contained freebase nicotine until the introduction of JUUL to the US marketplace in 2015 (Duell et al., 2020; Jackler & Ramamurthi 2019). Future studies would benefit from including freebase nicotine condition(s) as a direct comparison to protonated nicotine to further characterize the influence of nicotine protonation in liquids used with ECIG devices.

Finally, the laboratory setting may limit the generalizability of the present study. Future studies of protonated nicotine would benefit from naturalistic observations and ambulatory data collection to improve external validity.

Conclusions

This clinical laboratory study examined the influence of liquid nicotine concentration and device power setting on the physiological and subjective effects of ECIGs containing protonated nicotine. This study also included a preliminary examination of the extent to which experience with ECIGs influenced these outcome measures. The results demonstrated that the nicotine

delivery profile of ECIG products containing protonated nicotine is influenced by the nicotine concentration in the liquid, the power of the device, user experience and user behavior. Overall, plasma nicotine concentration was greatest in the highest liquid nicotine and device power condition (30 mg/ml;30 W) following ten puffs. Following the *ad lib* use period, significant differences in plasma nicotine concentration were not revealed across liquid nicotine concentration or device power setting. The influence of user experience (subjective profile) and user behavior (puff topography) may explain the absence of an effect of liquid concentration and/or device power following the *ad lib* use period. Specifically, decreased puff count and duration may have been adopted in order to titrate nicotine dose and/or in response to increased ratings of harshness. The interaction of ECIG device characteristics (e.g., liquid concentration and device power) and subsequent subjective profile and user behavior is an essential consideration for stakeholders interested in seeing ECIGs regulated effectively.

The nicotine delivery profile of ECIG products is influenced by the nicotine concentration in the liquid, the power of the device, user experience and user behavior. Importantly, these factors can influence one another, as evidenced by the changes in puff duration in conditions rated as more harsh in this study. For this reason, all these factors are important components in determining ECIG nicotine delivery to users' blood and brain and should be considered integral when considering effective ECIG regulations. Regulations comprised of an upper limit of liquid nicotine concentration (as in the EU; Kennedy et al., 2017) aim to limit the nicotine delivery and, further, reduce the abuse liability of ECIGs. However, the present findings suggest that nicotine delivery is influenced at least as much by device power as by liquid concentration. Additionally, puff duration can impact the nicotine delivery profile of ECIGs directly, suggesting that comprehensive regulations must include limits on puff duration.

Importantly, limiting puff duration is already a characteristic of popular ECIG products (JUUL, limit of 5.9 seconds; Karam et al., 2021). The results of the present study make clear that puff duration limits by themselves may not be effective in controlling nicotine delivery to the user and also highlight that a 5.9 sec duration limit is likely too long, as most participants took puffs that were much shorter. In order to construct comprehensive and effective ECIG regulations, the factors that influence nicotine delivery must be considered together; one way in which this can be achieved is combining regulations targeting nicotine flux (the rate at which nicotine is emitted from the ECIG; Eissenberg et al., 2020) and regulations that limit puff duration. By limiting nicotine emission rate and puff duration simultaneously, regulators may hope to gain control over the maximum dose of nicotine ECIG users can self-administer with each puff, independent of the nicotine concentration of the liquid and the form of the nicotine in the liquid (freebase vs protonated).

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Appendix A

Hughes-Hatsukami Withdrawal VAS Scale (Hughes & Hatsukami, 1986).

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.

	Not at all	**Extremely**
1. Urges to use a cigarette	———	
2. Irritability/frustration/anger	———	
3. Anxious	———	
4. Difficulty concentrating	———	
5. Restlessness	———	
6. Hunger	———	
7. Impatient	———	
8. Craving a cigarette/nicotine	———	
9. Drowsiness	———	
10. Depression/feeling blue	———	
11. Desire for sweets	———	

Appendix B

Direct Effects of Nicotine Scale

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.

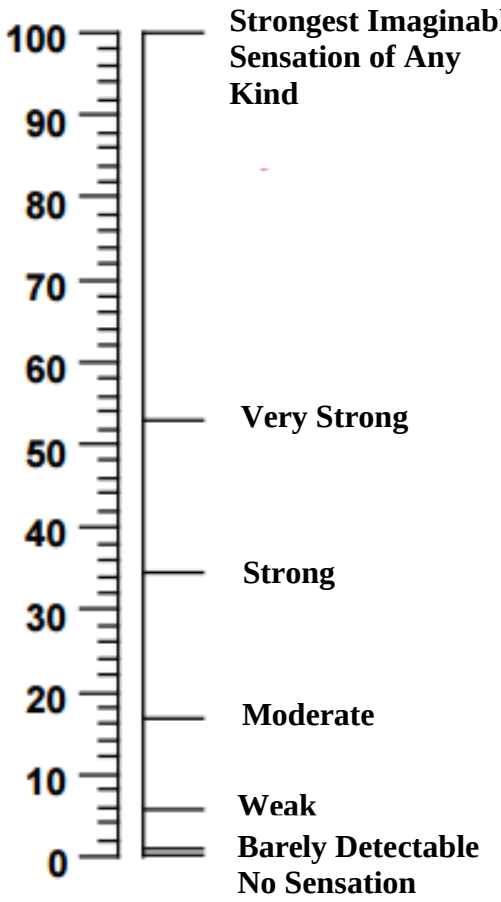
	Not at all	**Extremely**
12. Nauseous		
13. Dizzy		
14. Lightheaded		
15. Nervous		
16. Sweaty		
17. Headache		
18. Excessive salivation		
19. Heart pounding		
20. Confused		
21. Weak		

Appendix C

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?



Please specify the number you indicated with the horizontal line