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Modeling Drug Exposure in Rodents using e-Cigarettes and other Electronic Nicotine Delivery Systems

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Abstract

Smoking tobacco products is the leading cause of preventable death worldwide. Coordinated efforts have successfully reduced tobacco cigarette smoking in the United States; however, electronic cigarettes (e-cigarette) and other electronic nicotine delivery systems (ENDS) recently have replaced traditional cigarettes for many users. While the clinical risks associated with long-term ENDS use remain unclear, advancements in preclinical rodent models will enhance our understanding of their overall health effects. This review examines the peripheral and central effects of ENDS-mediated exposure to nicotine and other drugs of abuse in rodents and evaluates current techniques for implementing ENDS in preclinical research.

Keywords

e-Cigarette; ENDS; mouse; nicotine; rat; vaping

The Increased Prevalence of ENDS Use

The use of tobacco products represents one of the most expensive and detrimental health issues facing the world today. Cigarette smoking is a leading risk factor for a cardiovascular and respiratory diseases, causing 80% of deaths occurring from chronic obstructive pulmonary disease (COPD) (Tashkin and Murray, 2009) and increasing the risk for coronary heart disease and stroke by 2-4 times (FDA, 2012). Smoking is responsible for 90% of lung cancer deaths (FDA, 2012) and has been linked to cancer in nearly every organ of the body. Tobacco inhalation produces systemic inflammation and decreases immune function; thus, targeting specific immune processes altered by smoking represents an important therapeutic avenue for these disease states (Zuo et al., 2014). Overall, addiction to tobacco products

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Declaration of Competing Interest

The authors declare that they have no competing interests.

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remains the leading cause of preventable premature death worldwide (Tashkin and Murray, 2009). Persistent usage of tobacco-based products despite these negative health effects is largely due to the addictive properties of nicotine. However, even the most effective treatment strategies benefit only a fraction of all cigarette users, and most patients (60-80%) relapse within six months (Jorenby et al., 2006). Given the challenges of smoking cessation, substantial efforts have been directed toward discouragement of initial adolescent tobacco use through social stigma and implementation of government policy to reduce the incidence of future addicts.

While these approaches have curbed tobacco cigarette smoking significantly in the US, recent trends indicate that traditional cigarettes have been replaced by electronic cigarettes (e-cigarette) and other electronic nicotine delivery systems (ENDS) in young demographics (Figure 1), ultimately surpassing cigarette use in youths in 2014 (CDC, 2016; Gentzke et al., 2019). ENDS are battery-powered devices that heat and vaporize a liquid mixture containing a vehicle (e.g., propylene glycol, glycerol), drug (e.g., nicotine, 9-tetrahydrocannabinol), and/or aromatic flavorings (e.g., menthol, green apple, vanilla) which is inhaled by the user (vaping, or vapor inhalation/exposure). While these devices do not contain tobacco with its known characteristic carcinogenic agents, the overall health implications of long-term ENDS use are largely unknown (as discussed in multiple excellent reviews including but not limited to: (Benowitz and Burbank, 2016; Dautzenberg and Garelik, 2017; Dicipinigitis, 2017; Morjaria et al., 2017; Thirion-Romero et al., 2019). The dearth of this vital information renders it exceptionally difficult for individuals, healthcare providers, regulating bodies, and other organizations to make informed decisions about ENDS use, guidance, and policy (CDC, 2016). Nonetheless, impulsive regulations on ENDS may have unintended consequences on conventional cigarette use, as studies of dual users suggest that limiting ENDS nicotine content in an effort to reduce the reinforcing effects of e-cigarette use may instead compel dual users to return to conventional cigarettes (Pacek et al., 2019). Despite these mounting concerns, ENDS manufacturers now comprise a multi-billion-dollar industry for recreational nicotine consumption, and the popularity of their use by adolescents has reversed much of the progress made in the past decade toward reducing nicotine addiction in this demographic (Glantz and Bareham, 2018; McMillen et al., 2015). Accordingly, the rapid increase in ENDS use highlights the importance of developing a scientific, data-driven approach to understanding their potential health implications.

While ENDS have undergone a number of mechanical and stylistic changes since arriving on the market in the early 2000s, commercially available ENDS may be categorized into one of four generations of devices (Breland et al., 2017; Protano et al., 2018), as well as a new emerging Pod-style device (Huang et al., 2019). First-generation devices are disposable or rechargeable devices designed to mimic the look and feel of conventional cigarettes, and have come to be known as “cigalikes”. Second-generation devices resemble a pen or laser pointer, with increased battery capacity as compared to the first generation. These devices utilize a clear atomizer (“clearomizer”), which consists of a heating element inside of a clear tank of e-liquid. Third-generation devices include mechanical modification and variable voltage devices, the latter of which allows for the user to set the wattage or voltage of the device to customize the vaping experience and to change the properties of the vapor cloud produced. Fourth-generation devices include sub-ohm tanks and temperature control

devices. Pod-based systems popularized by JUUL provide an alternative to typical ENDS devices (Huang et al., 2019), and do not align neatly with any previous generations. Similar to first-generation devices, these do not allow for customization of heating power and other settings, so their popularity in the US market can be attributed largely to their relative convenience and ease of use for novice users (Galstyan et al., 2019). By eliminating the requirement to have e-liquid and refill tank on hand, users purchase closed cartridges or pods of e-liquid online or from local convenience stores as needed. The wide variability in ENDS devices manufacturing and quality control presents a substantial challenge in evaluating their potential hazard risks, as studies have shown that nicotine and toxicant exposure depend on a number of factors including coil resistance, heating method, and e-liquid composition (Kosmider et al., 2014; Mulder et al., 2019; Ooi et al., 2019; Talih et al., 2015). Likewise, these same parameters also change the aerosol particle size that drive the bioavailability of any substances found in the aerosol (Mulder et al., 2019). While the potential health risks posed by rapidly evolving configurations of ENDS remain unknown, hazards associated with many of the core components common to these devices such as drug content, vehicles and flavors merit substantial preclinical evaluation.

Preclinical Rodent Models of ENDS Use

Preclinical rodent models (Figure 2) provide an important tool in evaluating the health effects of nicotine and tobacco (FDA, 2012). Traditionally, the biological impact of nicotine in rodents has been studied using several routes of administration: intravenous (IV) self-administration, subcutaneous (SC) or intraperitoneal (IP) acute syringe injection, or continuous SC exposure by a surgically implanted osmotic minipump (Matta et al., 2007). Collectively, studies using these approaches suggest that the route of administration profoundly influences the behavioral and biological properties of nicotine. Inhalation remains the predominant method for nicotine and tobacco consumption in clinical populations. While a number of paradigms have been developed for evaluating tobacco and ENDS in rodents, these systems mainly fall into three categories (Figure 3): (1) Aerosol nebulizers, (2) Smoking Machines, and (3) Vacuum-based Vapor Systems. Ultimately, the optimal vapor exposure system will depend largely on the experimental question at hand.

(1) Aerosol Nebulizers:

Drug delivery by aerosol nebulization is well-established in clinical practice. This technique creates a mist of small droplets from an aqueous solution of drug that can be inhaled for rapid drug delivery. For rodent use, researchers have adapted aerosol nebulizers to create inhalable aerosol mist that contains nicotine and other flavor compounds. A number of commercially available aerosol nebulizers are employed in these studies including AeroDeliver (Espinoza-Derout et al., 2019a), Bioaerosol Nebulizer Generator (Ahmad et al., 2019), and AeroNeb (Schweitzer et al., 2015). This approach utilizes precisely controlled amount of drug delivery to the experimental animal and is highly effectual for the study of pharmacokinetic and behavioral parameters of inhaled nicotine. However, this configuration does not incorporate a heating element as is found in human ENDS, thereby limiting its utility for examining detrimental health effects caused by heat-induced chemical changes to the nicotine, vehicle, and aromatic flavorings.

(2) Smoking Machines:

Evaluation of the health effects of rodent cigarette smoke exposure was initially performed using Smoking Machines. A typical Smoking Machine recapitulates human cigarette inhalation using a heating element along with intermittently activated negative pressure created by a vacuum or peristaltic pump to generate cigarette smoke. The smoke is then passed via positive pressure to the rodent for inhalation. When administered through a nosecone to physically restrained rodents, smoking machines can produce precise amounts of nicotine and other drugs as they would be inhaled by a typical smoker from a given number of cigarettes. However, isolated restraint of rodents for extended periods of time induces stress, thereby confounding reliable evaluation of behavioral endpoints. To study the effect of smoking on rodent behavior, smoke administered into housing-like chambers recapitulate a secondhand smoking experience and allow rodents to eat, drink, and interact with their cage mates as usual. Many commercially available and homemade Smoking Machines have been adapted for ENDS, with the inExpose System being the most commonly used (Alasmari et al., 2018). Unlike Aerosol Nebulizers, the use of a heating element by Smoking Machines more accurately captures the chemical changes induced by rapid and repeated heating of nicotine e-Cigarette solutions that may exert adverse health effects in addition to those produced by the solution components. In addition, while Smoking Machines provide reliable dose-dependent administration of ENDS vapor, passage of ENDS vapor through the pump may necessitate regular cleaning and maintenance during long-term chronic exposure paradigms.

(3) Vacuum Exposure Systems:

In contrast with Smoking Machines, vacuum exposure systems incorporate the pump downstream of both the ENDS and the rodent exposure chamber. This setup creates a negative pressure similar to that of ventilated rodent housing racks, where the ambient air is constantly replaced with fresh air from outside the cage. Intermittent activation of the ENDS produces a heated drug vapor that is pulled into the rodent cage for inhalation, and subsequently aspirated out of the cage into a filter or trap system. Many commercially available and homemade Vacuum Exposure Systems have been developed for ENDS, with equipment from Scientific Vapor most commonly used to evaluate vapor exposure to drugs of abuse (Nguyen et al., 2016a; Nguyen et al., 2016b). This approach cannot be adopted for administration of vapor through nose-cones with restrained rodents; however, unrestrained animals may be group-housed and allowed to move without restriction for the duration of exposure with free access to food and water. Moreover, the components in direct contact with ENDS vapor (tubing, housing chamber) can be cleaned easily between uses. For these reasons, Vacuum Exposure Systems are an excellent tool for long-term studies of the behavioral effects of chronic vapor exposure.

Components of a Typical ENDS Vaping Solution

Vehicle:

ENDS vapor solutions (also known as e-liquids or e-juices) can produce biological and behavioral effects independent of nicotine. Nearly all commonly available e-liquids contain propylene glycol (PG) and vegetable glycerin (VG) as principal components, with these

chemicals comprising greater than 75% of the total ENDS vapor solution (Kosmider et al., 2014). A clinical study recently conducted using different PG/VG ratios in smokers blinded to treatment group showed that the relative ratio does not affect the reinforcement value (Smith et al., 2019). Heated e-liquids have been found to release potentially toxic chemicals such as formaldehyde, acetaldehyde, acetone, and acrolein (Kosmider et al., 2014; Rubinstein et al., 2018). The Institute of Medicine previously ranked acetaldehyde, acrolein, and formaldehyde as the most significant cardiovascular toxins in tobacco smoke (NAP, 2010), as these aldehydes are estimated to comprise 92% of the clinical risk for cardiopulmonary (*i.e.*, noncancer-related) disease from smoking (Hausmann, 2012). Toxic aldehydes are also present in e-cigarette aerosols (Bertholon et al., 2013; Kosmider et al., 2014). Although levels of these constituents are less than those generated from standard cigarettes (Farsalinos et al., 2017), newer e-cigarette devices have higher battery power output which dramatically increases the release of aldehydes due to overheating of the coil (Ogunwale et al., 2017). The voltage coil values influence the amounts of toxic aldehydes produced, leading to harmful toxicological effects (Cirillo et al., 2019). At present, the prevailing view is that ENDS possess lower carcinogenic risk than traditional cigarettes, yet the emerging trend toward development of more powerful devices may diminish this gap over time.

Evidence suggests that in the absence of nicotine or other drugs of abuse, non-aerosolized e-liquids do not appear to exhibit significant abuse liability in rodent models. Rats exhibit similar preference for nicotine solubilized in saline or e-liquid under the intravenous self-administration (IVSA) paradigm, and there is no difference in their dose-effect responses to nicotine with either solution (LeSage et al., 2016; Smethells et al., 2018). Systemic exposure to e-liquids without nicotine do not alter intracranial self-stimulation (ICSS) thresholds (Harris et al., 2018a), and only attenuate nicotine-induced changes in ICSS at the highest doses of nicotine exposure (Harris et al., 2017; Harris et al., 2018b; LeSage et al., 2016). In addition, there is no difference in mecamylamine-precipitated nicotine withdrawal between groups receiving chronic SC administration of either nicotine alone or e-liquid solutions containing nicotine by osmotic minipump (Harris et al., 2017), demonstrating that nicotine alone is responsible for the development of dependence using this route of exposure. However, e-liquids are not completely inert as they produce a modest attenuation of nicotine-induced diminishment of food intake (Bunney et al., 2018), as well as low-affinity binding to $\alpha 4\beta 2$ nicotinic cholinergic receptors (nAChRs) (Harris et al., 2018b). Additionally, IP administration of e-liquid can impair working memory as assessed using the novel object recognition task and spontaneous alternation T-Maze task (Golli et al., 2016). Collectively, these studies suggest that the solvent constituents of e-liquids do not inherently possess robust psychoactive properties. However, the majority of studies published to date do not include proper vehicle controls, so future work should address if ENDS produce chemical changes in or altered pharmacokinetic properties of e-liquids that ultimately enhance their abuse liability.

Nicotine Content:

The vapor solution concentration is typically expressed as either the percentage (ml of nicotine per ml) or concentration (mg nicotine per ml) of nicotine per total volume of e-

liquid. With a density of approximately 1 g/ml, a 20 mg/ml e-liquid would contain the same amount of nicotine as a 2% e-liquid. Most e-liquids available for consumer purchase contain between 0-30 mg/ml nicotine, and all the rodent ENDS research identified by our search strategy utilized the same concentration range. An evaluation of 35 popular e-juices and refill cartridges determined that nearly half were inaccurately labeled for nicotine content (Goniewicz et al., 2013), so likely it will be difficult to establish an optimal human dose preference based on brand labels. Likewise, the company JUUL has recently popularized nicotine salt solutions with elevated nicotine levels in e-liquids up to 60 mg/ml, suggesting that future configurations may continue to increase the nicotine content of e-liquids.

Selecting the appropriate exposure dosing paradigm remains one of the significant challenges for performing ENDS exposure experiments in rodents. In humans, blood plasma concentrations in smokers generally range from 10-50 ng/ml, with concentrations as high as 100 ng/ml in some cigarette users. A recent evaluation of ENDS usage in people demonstrated that despite the wide range of nicotine concentrations in their preferred e-liquids (4.1-15.3 mg/ml), users instinctively altered their inhalation duration and intensity to achieve similar peak levels of nicotine in a short vaping session as achieved by smoking a single cigarette (St Helen et al., 2016). In humans, nicotine is metabolized by the liver at a relatively slow rate ($T_{1/2}$ =120 min) when compared with rats ($T_{1/2}$ =45 min) and mice ($T_{1/2}$ =6 min) (Matta et al., 2007). Likewise, assessment of the plasma cotinine ($T_{1/2}$ =45 min), the primary stable metabolite of nicotine, is routinely used in clinical populations as an index of nicotine exposure (Benowitz, 2009). However, cotinine metabolism in both rats ($T_{1/2}$ =25-50 min) (Craig et al., 2014; Kyrematen et al., 1988) and mice ($T_{1/2}$ =25-50 min) (Li et al., 2013; Siu and Tyndale, 2007) is substantially accelerated as compared with humans. Importantly, the route of administration may strongly influence nicotine pharmacokinetics as recent studies demonstrate that ENDS exposure may extend its half-life in rats ($T_{1/2}$ =4 hrs) (Shao et al., 2018) and mice ($T_{1/2}$ =2 hrs) (Shao et al., 2019). Given the disparities in nicotine metabolism between species (particularly in mice), the dosing requirements for eliciting nicotine reward and dependence in rodents using ENDS may vary greatly from the parameters found in clinical populations.

Flavoring:

ENDS users commonly supplement e-liquid vapor solutions with aromatic flavorings to enhance the experience of vaping. These solutions can be divided into three main flavoring categories: regular (tobacco), menthol, and flavored (green apple, vanilla, etc.) (Kuiper et al., 2018). In a survey of current users between the ages of 12 and 17 years old, 81.5% of responders reported that they use e-cigarettes “because they come in flavors I like” (Ambrose et al., 2015). Six out of the twenty-four most common flavor chemicals (including vanillin) are aldehydes, which are ‘primary irritants’ of mucosal tissue (Tierney et al., 2016). Accordingly, *in vitro* assessment of human and mouse bronchial epithelial cells demonstrated that five of the seven common aromatic flavoring chemicals including vanillin lead to cytotoxicity at concentrations typically found in e-liquids (Sherwood and Boitano, 2016). Menthol administered IP hinders nicotine clearance (Alsharari et al., 2015), alters nicotine-induced behaviors (Henderson et al., 2016), enhances nicotine withdrawal (Alsharari et al., 2015), upregulates nicotinic cholinergic receptors (nAChR) after chronic

treatment (Alsharari et al., 2015; Henderson et al., 2017; Henderson et al., 2016), and alters the firing rate of dopaminergic neurons (Henderson et al., 2017; Henderson et al., 2016). Additionally, menthol increases oral nicotine consumption in male rats (Haskins et al., 2018). While some groups report potential sex-specific responses to menthol (Alsharari et al., 2015; Bagdas et al., 2019), others suggest that menthol alters reward circuitry in both sexes (Henderson et al., 2017; Henderson et al., 2016). Furthermore, menthol can reduce the aversive effects of nicotine in a two-bottle choice model of conditioned taste aversion in adolescent rats of both sexes (Harris et al., 2019), suggesting that certain flavors may facilitate increased consumption of nicotine in young adults. Likewise, Farnesol, a commonly used chemical in fruity e-liquids including Green Apple, facilitates reward-like behaviors including condition place preference (CPP), increases the firing rate of dopaminergic neurons in the ventral tegmental area (VTA), and upregulates $\alpha 6$ -containing nAChRs following IP administration in male mice (Avelar et al., 2019). Collectively, these studies suggest that ENDS flavorings may contribute to reward in a sex-specific manner. It is critical to note that while some flavor chemicals may be FDA-approved as safe for ingestion in certain amounts, there is a substantial gap in knowledge regarding the safety and bioactivity of these chemicals when inhaled using ENDS.

Peripheral Effects of Nicotine Vapor Exposure

Chronic tobacco use driven by nicotine dependence produces adverse health consequences via exerting effects on several organ systems outside of the central nervous system. These include, but are not limited to, respiratory (Lee et al., 2007; Sekhon et al., 1999), cardiovascular (CV) (Ambrose and Barua, 2004; Viridis et al., 2010), gastrointestinal (GI) (Chi et al., 2017; Shin et al., 2005), and immune systems (Geng et al., 1995; Sopori et al., 1998). The relative risks of chronic ENDS use on these structures remain to be clarified, and elucidating immediate and long-term effects of vaping among young demographics is of paramount importance. Although initial studies suggest that the many of the harmful effects classically associated with smoking traditional cigarettes appear to be reduced when switching to e-cigarettes (Bertholon et al., 2013; Hwang et al., 2016; Stewart et al., 2018; Xie et al., 1997; Yan and D'Ruiz, 2015), recent reports suggest that ENDS subject the user to a unique set of health hazards (CDC, 2016; FDA, 2019). This year, the CDC has released alarming reports of up to possible 215 cases of severe pulmonary disease in ENDS users that exhibits symptoms distinct from those associated with traditional cigarettes (Schier et al., 2019). While at present these clinical pathologies have not been linked definitively to ENDS, increasing numbers of patient reports submitted to the FDA indicate a strong likelihood of adverse health effects from use of these devices. In light of these findings, rodent models of ENDS use likely will provide insight into the potential hazards of chronic nicotine vapor exposure. In this section, we will highlight seminal studies evaluating usage of ENDS in rodent models and discuss critical knowledge gaps with potential relevance to human health.

Cancer:

Human tobacco use is associated with increased risk of various cancers (Peerschke and Ghebrehwet, 2014). In 2011, cigarette smoking was responsible for 48.5% of deaths from 12 types of cancer combined including lung, oral cavity and throat, esophagus, larynx, acute

myeloid leukemia, stomach, pancreas, colon and rectum, bladder, kidney, and cervical (Siegel et al., 2019). The greatest percentage of smoking deaths are derived from lung cancer (80.2%) and larynx cancer (76.6%) (Siegel et al., 2019). Cancer from smoking has been attributed to various hazardous chemicals present in tobacco smoke, at least 69 of which are considered carcinogenic (CDC, 2014; NTP, 2016). Conversely, evidence in support of a direct link between nicotine exposure and cancer risk was deemed “inadequate” by the Surgeon General in 2014 (CDC, 2014). This report was published less than 8 years after e-cigarettes were introduced to the United States, likely before significant data on ENDS use had accumulated. In human urine samples of ENDS users, two carcinogenic compounds (o-toluidine and 2-naphthylamine) have been detected at high concentrations, suggesting a potential risk of cancer development from e-cigarettes (Fuller et al., 2018).

Only a limited number of studies have investigated the effects of ENDS use on DNA damage. Following 4 weeks of ENDS exposure, male rats exhibit a decrease in the percentage of double-stranded DNA in white blood cells (Vivarelli et al., 2019), along with elevated levels of oxidative stress markers in lungs (Canistro et al., 2017) and testes (Vivarelli et al., 2019). In mice, 12 weeks of nicotine vapor exposure leads to increased oxidative stress markers as well as oxidative DNA damage in the liver (Espinoza-Derout et al., 2019b; Hasan et al., 2019). Furthermore, in a comprehensive analysis of vapor exposure, Lee *et al.* showed that 12 weeks of ENDS use reduces DNA repair in the lung, and induces DNA damage in mice in the lung, bladder, and heart (Lee et al., 2018). Collectively, these reports suggest that long-term ENDS use may contribute to lung cancer, although the overall risk relative to cigarette smoking remains fiercely debated (Lee et al., 2018; Li Volti et al., 2018; Queimado et al., 2018; Tang, 2018a, b). Given the proposed use for ENDS as a medical device (Pourchez et al., 2017; Varlet et al., 2016), additional well-controlled studies are required in order to settle the controversy surrounding a causal link to cancer development.

Cardiovascular System:

Cigarette smoking damages the CV system and augments the risk of heart disease (CDC, 2014). Specifically, tobacco smoke produces increased blood pressure and heart rate, altered cholesterol and triglycerides levels, and damage to blood vessel walls. These changes can promote accumulation of plaques in the arteries (atherosclerosis) and narrowing of blood vessels, causing damage to the heart and impeding delivery of oxygen to the body. Likewise, clinical studies evaluating the cardiovascular effects of ENDS use have shown that vaping produces elevated heart rate and diastolic blood pressure (Nides et al., 2014; Yan and D’Ruiz, 2015) as well as increased aortic stiffness, similar to traditional cigarette smoking (Vlachopoulos et al., 2016). These adverse effects have been linked to increased risk of atherosclerotic heart disease, myocardial infarction, heart failure, and stroke (Sethi et al., 2014).

Importantly, rodent models of ENDS capture many of the CV effects also observed in nicotine-dependent human populations. Two independent studies detected increased expression of cholesterol-related genes in the liver (Hasan et al., 2019; Phillips et al., 2017) and elevated cholesterol levels in the blood (Canistro et al., 2017; Phillips et al., 2017),

following chronic ENDS exposure. Changes in blood levels of triglycerides remain equivocal, as two studies find that they are elevated (Canistro et al., 2017; Hasan et al., 2019) while another reports decreased amounts following ENDS use (Phillips et al., 2017). Acute exposure to nicotine vapor also has been shown to reduce heart rate in rats (Ahmad et al., 2019). However, there is some disagreement regarding chronic ENDS use in rodents; *e.g.*, some studies demonstrate reduced heart rate (Crotty Alexander et al., 2018) while others observe no significant change in this parameter (Olfert et al., 2018; Orzabal et al., 2019). Repeated nicotine ENDS exposure for 1 week causes thrombogenesis in mice, with decreased bleeding time and time to occlusion with tail bleed (Qasim et al., 2018). This phenomenon results in part from increased platelet reactivity, with increased responsivity to pro-thrombotic agents ADP and U46619 and resistance to anti-thrombotic prostacyclins. Echocardiogram analysis demonstrates that chronic ENDS exposure leads to cardiac dysfunction in mice, wherein a trend for diminished ejection fraction at week 2 (Shi et al., 2019) then becomes significantly reduced at week 12 (Espinoza-Derout et al., 2019a) and week 35 (Olfert et al., 2018). Concomitant changes in cardiac fractional shortening also are observed at weeks 12 and 35 (Espinoza-Derout et al., 2019a; Olfert et al., 2018). Additionally, chronic ENDS use increases blood pressure (Crotty Alexander et al., 2018), cardiac and hepatic fibrosis (Crotty Alexander et al., 2018; Espinoza-Derout et al., 2019a), and atherosclerotic lesion formation (Espinoza-Derout et al., 2019a). While ENDS appear to exert reduced effects on cardiac function compared with cigarette smoke in mice, vaping produces aortic stiffness and an overall predicted magnitude of aortic vascular dysfunction that is equivalent to long-term cigarette smoking (Olfert et al., 2018).

Respiratory System:

Both cigarette smoking and ENDS use negatively affect overall respiratory function. Chronic cigarette use increases the risk of lung cancer and causes chronic obstructive pulmonary disease (COPD) (Siegel et al., 2019). Two independent groups have linked e-cigarette use with the development of COPD (Perez et al., 2019; Wills et al., 2019), with ENDS producing a similar risk to cigarette smoking with adjusted odds ratios (AOR) of 2.58 vs. 2.98, respectively. Likewise, both ENDS use (AOR 1.33) and cigarette smoking (AOR 1.37) show significant positive correlation with the development of asthma. Smoke-induced airway irritation and cough associated with cigarettes similarly are induced by inhalation of nicotine aerosols (Lee et al., 2007), implicating a nicotine-dependent mechanism for these dysfunctions. Accordingly, the CDC has summarized reports of up to 215 possible cases of severe pulmonary disease associated with ENDS use (Schier et al., 2019). While a direct link has not yet been established, this year the FDA has begun soliciting patient reports that may provide sufficient evidence for the potential pulmonary health risks of ENDS use.

Studies conducted in rodent models suggest that ENDS-induced impairment of respiratory function may develop through mechanisms distinct from those produced by cigarette exposure. While in rodents cigarette smoke produces visible lung injury that is readily assessed by hematoxylin and eosin staining (Feldman and Wolfe, 2014), multiple studies failed to observe the same magnitude of damage by ENDS following exposure for 1 week (Glynos et al., 2018; Husari et al., 2016), 4 weeks (Glynos et al., 2018; Hwang et al., 2016), or 8 weeks (Larcombe et al., 2017). While this pathology is detectable at 17 weeks of

vaping, exposure to cigarette smoke leads to lung damage at a substantially faster rate in rodent models. However, nicotine vaping by ENDS does trigger airway hyper-responsiveness as early as the first week of exposure (Glynos et al., 2018), and continued usage maintains this enhanced reactivity for at least 8 weeks (Garcia-Arcos et al., 2016). Additionally, 8 weeks of ENDS exposure leads to multiple additional impairments in pulmonary function not observed in mice subjected to cigarette smoke (Larcombe et al., 2017) that are maintained at 17 weeks of ENDS use (Garcia-Arcos et al., 2016). Collectively, these studies indicate that chronic nicotine vapor exposure can produce hallmark features of COPD which may be achieved through mechanisms other than cigarette smoking.

Immune System:

Reports outlining effects of tobacco smoking on immune function are seemingly contradictory, with cigarettes producing both pro-inflammatory and immunosuppressive effects simultaneously (de Heens et al., 2009; Stampfli and Anderson, 2009). Cigarette smoke contains more than 4500 chemical components, and many of these reactive species trigger the release of inflammatory cytokines from epithelial cells that lead to inflammatory disorders of the lung and other organs (Bhalla et al., 2009; Bozinovski et al., 2016). Likewise, acute exposure to e-liquid vapor without nicotine causes increases in cardiovascular inflammatory markers including C-reactive protein and nitric oxide metabolites (Chatterjee et al., 2019), demonstrating that both cigarettes and ENDS can trigger an immediate immune response. In contrast, nicotine itself can activate nicotinic receptors (specifically $\alpha 7$, and possibly others) on macrophages and other immune cells to attenuate cytokine release (Arnson et al., 2010). This direct immunosuppressive action of nicotine may provide some explanation for the perplexing negative correlation of smoking with inflammatory neurological disorders such as Parkinson's disease, multiple sclerosis, and Alzheimer's Disease (Piao et al., 2009). Chronic cigarette exposure can suppress Th1 and Th17 immune responses (Piao et al., 2009), yet current evidence suggests that it drives the immune system in the direction of the Th2 pathway (Sopori, 2002; Stampfli and Anderson, 2009).

Chronic ENDS exposure in rodents produces changes in interleukin-6 (IL-6) that mirror clinical findings in tobacco smokers. In chronic smokers, a history of cigarette smoking correlates with increased IL-6 release in plasma (McEvoy et al., 2015; Sunyer et al., 2009) and exhaled breath condensate (Carpagnano et al., 2003). Results from limited clinical studies of ENDS use demonstrate that IL-6 is elevated in saliva (Fritz and Weissbach, 1985) and in peri-implant crevicular fluid (BinShabaib et al., 2019). In mice, short-term ENDS exposure for 3 days augments levels of IL-6 in bronchoalveolar lavage fluid (BALF) (Glynos et al., 2018; Husari et al., 2016) which remain elevated during long-term, multi-week exposure (Garcia-Arcos et al., 2016). The relative impact of nicotine and e-liquid vapor on IL-6 remains unclear, as some report conflicting evidence and others fail to properly control for the effect of e-liquid vehicle. For example, alveolar macrophages collected from smokers display attenuated IL-6 release (Mikuniya et al., 1999; Soliman and Twigg, 1992), while exposure of human bronchial epithelial cells to either nicotine or e-liquid can induce IL-6 production (Ahmad et al., 2019; Garcia-Arcos et al., 2016; Wu et al.,

2014). Consistent with the hypothesis that smoking and ENDS facilitate a Th2-dependent response, IL-6 can promote Th2 differentiation while also inhibiting Th1 polarization (Diehl and Rincon, 2002).

Downstream cytokine activation profiles following exposure to ENDS in rodents provide further support of enhanced Th2 responsivity. While the classic nomenclature does not fully capture our current understanding of the roles of endogenous cytokines which may depend on cellular context, Th1 cells release the “pro-inflammatory” cytokines tumor necrosis factor alpha (TNF α) and interferon gamma (IFN γ), whereas Th2 cells produce cytokines generally considered to be “anti-inflammatory” including interleukin-4 (IL-4) and interleukin-13 (IL-13) following activation (Leung et al., 2010). Nonetheless, in contrast to their “anti-inflammatory” role, IL-4 and IL-13 also participate in driving pulmonary allergic reactions (Gour and Wills-Karp, 2015). Chronic 10-week administration of e-cigarette solution containing nicotine directly into the trachea produces increased levels of both IL-4 and IL-13 in mice (Lim and Kim, 2014), and 16 weeks of ENDS elevated IL-4 in the mouse larynx tissue to levels similar to those following cigarette smoke exposure (Ha et al., 2019). However, most studies evaluating the effect of ENDS on TNF α and IFN γ failed to identify significant changes in these Th1-dependent cytokines in serum (Zelikoff et al., 2018), larynx (Lim and Kim, 2014), lung (Glynos et al., 2018; Husari et al., 2016), or BALF (Hwang et al., 2016; Larcombe et al., 2017; Phillips et al., 2019). Following 3-6 months of ENDS, serum from C57BL/6 mice showed elevated IFN γ but diminished TNF α ; concomitant analyses of plasma from outbred CD1 mice showed no change in either cytokine, suggesting Th1 responsivity to ENDS vapor may have a genetic component (Crotty Alexander et al., 2018). Moreover, two studies that identified elevated TNF α levels in the plasma (Kaisar et al., 2017) and lung (Chen et al., 2018a) following ENDS use showed similar results using nicotine-free e-liquid, suggesting that Th1 responsivity may occur independently of nicotine. Taken together, these findings support a Th2-driven phenotype in response to chronic ENDS use. Future work should expand cytokine analyses to further characterize the immune response to vapor exposure of nicotine versus vehicle controls.

Metabolism and Body Weight:

Body weight control remains an important reason for smokers to continue using tobacco products (Fulkerson and French, 2003; Mineur et al., 2011), and post-cessation weight gain can facilitate relapse. It is well-documented that nicotine affects energy metabolism by acting on both the peripheral (Filozof et al., 2004; Jo et al., 2002) and central (Martinez de Morentin et al., 2012; Mineur et al., 2011; Tweed et al., 2012) nervous systems. Specifically, peripheral nicotinic receptors in the adrenal glands stimulate the release of vascular catecholamines that produce insulin resistance, impair glucose tolerance, and alter other metabolic responses (Audrain-McGovern and Benowitz, 2011; Benowitz, 2003; Ferrara et al., 2001; Filozof et al., 2004; Tweed et al., 2012; Wu et al., 2015). Moreover, nicotine acts on adipocytes to induce lipolysis and elevate free fatty acids levels in the blood, exacerbating the effect of nicotine on insulin resistance (Wu et al., 2015) and enhancing thermogenesis in brown adipose tissue (Stojakovic et al., 2017). In the brain, nicotine regulates satiety-related peptidergic neurons in the hypothalamus to alter food intake and energetic expenditure (Huang et al., 2011; Hussain et al., 2012; Mineur et al., 2011). Additionally, there is

considerable cross-talk between peripheral and central signaling systems; for example, nicotine-induced orexigenic and anorexigenic peptidergic activities in the hypothalamus drive changes in peripheral circulating levels of leptin and ghrelin in the blood (Bouros et al., 2006; Dickson et al., 2010; Hussain et al., 2012; Stojakovic et al., 2017). As a cumulative result of these processes, smokers tend to have lower body weight than non-smokers (Mineur et al., 2011), while protracted abstinence from smoking leads to body weight gain (Audrain-McGovern and Benowitz, 2011; Filozof et al., 2004; Jo et al., 2002). For these reasons, both non-smokers (Lanza et al., 2017; Morean and Wedel, 2017) and current cigarette smokers (Russo et al., 2018) consider ENDS as a method for body weight control. However, no studies have systematically evaluated the effects of ENDS on metabolism in clinical populations.

The majority of preclinical studies show that ENDS use leads to decreased body weight in rodents independent of the apparatus used to expose them (Chen et al., 2018a; Larcombe et al., 2017; McGrath-Morrow et al., 2015; Orzabal et al., 2019; Oviedo et al., 2016; Phillips et al., 2019; Shao et al., 2019; Shi et al., 2019; Smith et al., 2015; Werley et al., 2016). In some cases, exposure to ENDS vehicle without nicotine also leads to changes in body weight (McGrath-Morrow et al., 2015; Smith et al., 2015), demonstrating that vapor inhalation itself may produce unanticipated metabolic effects. Conversely, some studies fail to observe ENDS-dependent changes in body weight (Chen et al., 2018b; Espinoza-Derout et al., 2019a; Lauterstein et al., 2016; Ponzoni et al., 2015; Vivarelli et al., 2019), suggesting that specific aspects of experimental design may influence changes in metabolism. Similar to humans, nicotine exposure with ENDS in rodents significantly alters lipid and cholesterol homeostasis in the blood (Oviedo et al., 2016), leading to increased activity of some enzymes in the liver (Oviedo et al., 2016; Phillips et al., 2015; Wong et al., 2016). Overall, ENDS-dependent changes in body weight likely reflect broadscale systemic changes in energy metabolism.

Central Nervous System Effects of Drug Vapor Exposure

Originally, ENDS were developed and promoted as a lower risk alternative to smoking traditional tobacco cigarettes, and even have been proposed as a potential medical device to facilitate quitting. Likewise, the use of these devices as innovative tools for therapeutic drug delivery has been investigated (Breitbarth et al., 2018; Varlet et al., 2016). However, ENDS also have been used for recreational purposes by teenagers and young adults with nicotine-free or nicotine-containing solutions (CDC, 2016; Gentzke et al., 2019; Leventhal et al., 2015), as well as with illegal substances (Blundell et al., 2018; Breitbarth et al., 2018). According to preliminary results from the FDA, more than 1 in 4 high school students have used ENDS in the past 30 days, with menthol or fruit flavors such as Green Apple clearly standing out as the most popular additives (FDA, 2019). According to CDC Director Dr. Robert. R. Redfield (CDC, 2019), “The skyrocketing growth of young people’s e-cigarette use over the past year threatens to erase progress made in reducing youth tobacco use. It’s putting a new generation at risk for nicotine addiction.” Furthermore, US health officials are investigating the possibility that ENDS trigger seizures in susceptible populations, as over 100 patient reports indicate a potential link to ENDS use (Zolot, 2019). In light of these recent developments, ENDS represent an important tool for administration of several drugs

of abuse in rodents, thereby recapitulating human modes of consumption and lending translational relevance to preclinical animal models (MacLean et al., 2017).

Nicotine:

Nicotine addiction is a complex behavioral phenomenon, characterized by alterations in synaptic transmission which contribute to a progression of addiction-related processes including reward, dependence, and relapse (Koob and Volkow, 2010; Mao and McGehee, 2010; Russo et al., 2010). The motivational and addictive properties of nicotine are critically reliant on activation of nicotinic acetylcholine receptors (nAChRs) in the brain, and most therapeutic approaches for smoking cessation have focused on this aspect of nicotine signaling (Benowitz, 2009; Polosa and Benowitz, 2011). Three different clinical assessments reveal that dual users of both cigarettes and ENDS continue to display signs of nicotine dependence after one year (Piper et al., 2019). Concentration of nicotine in the e-liquid did not predict acute nicotine plasma concentrations (St Helen et al., 2016) or long-term nicotine dependence (Piper et al., 2019), suggesting that ENDS users alter their intake (puff intensity, duration, interpuff interval) while vaping to achieve a desired level of nicotine similar to that of cigarette consumption. Ultimately, differences in nicotine intake parameters may alter the addictive liabilities associated with ENDS as compared to conventional cigarettes.

Clinical evaluations suggest that consumption patterns of ENDS differ from typical cigarette use (St Helen et al., 2016). Consumption of cigarettes is fairly consistent between smokers, with a single cigarette typically puffed about 10-15 times in 5-8 minutes and the interval between cigarettes as indicative of the level of dependence (Benowitz et al., 2006). In contrast, the duration of a typical ENDS puff (3.5 seconds) is longer than a standard cigarette and the vaping patterns are more variable (St Helen et al., 2016). During 90 minutes of *ad libitum* access to ENDS, participants took approximately 5 unclustered single puffs, 9 short clusters (2 to 5 puffs), 2 medium clusters (6 to 10 puffs), and 1 long cluster (>10 puffs). On average, users had an interpuff interval of approximately 2 minutes. While the total amount of nicotine delivered during a 90-minute vaping session roughly equals about 3-4 tobacco cigarettes, ENDS users expressed blood plasma levels of nicotine similar to those expected from consumption of a single cigarette.

Rodent exposure to nicotine using ENDS also leads to substantial changes in energetic function in the brain. Following one week of exposure, mice demonstrate a reduction in glucose metabolism resulting from decreased expression of glucose transporters GLUT1 and GLUT3 (Sifat et al., 2018). Likewise, chronic nicotine vapor exposure significantly alters lipid metabolism in the brain. A comprehensive lipidomics analysis of the rat brain identified changes in the fatty acid profile at 8 weeks (Cardenia et al., 2018), most notably an increase in saturated fatty acids concomitant with reduced levels of polyunsaturated fatty acids as well as cholesterol and oxysterols. Collectively, these lipid changes in the brain indicate that ENDS use substantially increases risk of atherogenic and thrombogenic events. In addition, ENDS exposure of nicotine reduces body temperature in both rats (Javadi-Paydar et al., 2019) and mice (Lefever et al., 2017a), an effect that is prevented by mecamylamine, suggesting that nicotine directly mediates these responses *in vivo*. Route of administration-dependent sex differences in this temperature responsivity are detected in

mice (Lefever et al., 2017a), with females showing greater hypothermia following vapor exposure and males showing greater hypothermia following SC injection.

Chronic ENDS use in rodents also facilitates substantial alterations in molecular signaling pathways of the reward system. Expression of nicotinic receptor subunits $\alpha 4$ and $\beta 2$ is elevated after 7 weeks of ENDS exposure in multiple mesocorticolimbic regions including the nucleus accumbens, dorsal striatum, hippocampus, and cortex of mice (Ponzoni et al., 2015). These changes are similar to those found in mice exposed to cigarette smoke for 7 weeks. Long-term nicotine vapor exposure leads to plasticity in the nucleus accumbens, where mice show enhanced expression of FosB and increased AMPA GluA1/GluA2-3 receptor ratio (Ponzoni et al., 2019). ENDS exposure for six months alters glutamate regulatory systems including decreases in cystine/glutamate antiporter (xCT) and glutamate transporter 1 (GLT1) in the striatum (Alasmari et al., 2017). Consistent with other models of dependence, post mortem brain tissue analysis in mice exposed for 6 month to ENDS showed increased striatal glutamine/glutamate and decreased dopamine content, whereas tissue GABA levels were diminished in the frontal cortex (Alasmari et al., 2019). No differences in serotonin levels were identified in these brain regions. Taken together, these changes suggest a rewiring of the reward pathway following chronic nicotine inhalation, although further investigation using *in vivo* microdialysis or electrophysiology are essential to adequately evaluate changes in synaptic plasticity.

Behavioral responses to nicotine vapor exposure support activation and alteration of reward pathways. Acute ENDS exposure in male rats increases spontaneous locomotor activity caused by nicotine (Javadi-Paydar et al., 2019), as locomotor behavior is attenuated by the nicotinic receptor antagonist mecamylamine and is absent following vaping with vehicle e-liquid. In mice, both acute vapor exposure and SC injection of nicotine produce dose-dependent decreases in spontaneous locomotor in both sexes that are attenuated by pretreatment with mecamylamine (Lefever et al., 2017a). Furthermore, chronic ENDS exposure can produce behavioral changes that capture multiple aspects of nicotine dependence. In contrast with the effect of a single ENDS dosing session in mice (Lefever et al., 2017a), locomotor activity increases post-session following 10 weeks of chronic ENDS exposure (Shao et al., 2019). Following 7 weeks of exposure to nicotine vapor, mice express multiple signs of dependence during spontaneous abstinence, including decreased open arm time on the elevated plus-maze, increased marble burying time, and increased somatic symptoms observed (Ponzoni et al., 2015). These behavioral maladaptations are detected as late as 30 days into abstinence and are of similar magnitude to those produced by exposure to cigarette smoke. In contrast to other rodent studies (Javadi-Paydar et al., 2019; Lefever et al., 2017a; Shao et al., 2019), this group found no changes in locomotor activity suggesting that different ENDS equipment configurations as well as experimental conditions may capture distinct aspects of nicotine behavior in rodents. Collectively, these studies demonstrate that nicotine exposure by ENDS produces pharmacologically relevant behavioral responses, thereby offering critical information about the rewarding (and potentially the addictive) aspects of nicotine. However, future studies are needed to evaluate the molecular and behavioral impact of repeated ENDS exposure on the CNS when compared with more traditional preclinical exposure models.

Cannabinoids:

The legalization of cannabis for both therapeutic and recreational use has driven a dramatic expansion of cannabis products available for human consumption, which are particularly popular among young demographics (Trivers et al., 2018). The public perception that vaping cannabis extracts provides a safer alternative to smoking marijuana, along with the increased commercial availability of cannabis extracts (Spindle et al., 2019), has led to widespread usage of ENDS for cannabis delivery (Etter, 2015; Giroud et al., 2015; Morean et al., 2015). These extracts contain high concentration of cannabinoids, with levels of the main psychoactive compound Δ^9 -tetrahydrocannabinol (THC) typically between 50-90% of the total cannabinoid content (Meehan-Atrash et al., 2017) along with additional bioactive substances such as cannabidiol (CBD) and a wide range of terpenes and terpenoids (Varlet, 2016). Many marijuana dispensaries provide edible products with defined levels of THC and CBD for medicinal purposes; however, many customers choose inhalation as their preferred route of exposure for both medical and recreational use. A comparison between smoked and vaporized cannabis by human users suggests that ENDS result in faster absorption, stronger drug effects, and greater psychomotor and cognitive impairments (Abrams et al., 2007; Spindle et al., 2018). Likewise, young adults show a strong preference for vaping and/or dabbing (single breath from a water pipe) over smoking traditional marijuana cigarettes (Alzghari et al., 2017; Carliner et al., 2017) as these modes of delivery can produce high THC levels unachievable by smoking (Hadener et al., 2019) which may increase the addictive properties of cannabis.

Along with technological advances for human inhalation of cannabis, corresponding preclinical studies have incorporated these devices in the evaluation of effects of cannabinoids on rodents. Prior to the widespread availability of ENDS, acute effects of cannabinoid inhalation in rodents have been evaluated using aerosol nebulizers (Lichtman et al., 2000), metered dose inhalers (MDIs) (Wilson et al., 2002), and Volcano© vaporizer (Hazekamp et al., 2006; Hlozek et al., 2017; Manwell et al., 2014a; Nguyen et al., 2016b). Collectively, these studies show that vapor has a rapid onset and short-lasting behavioral effects compared to the other systemic routes of administration (IP, SC). In rats, vapor exposure to cannabinoids leads to an immediate peak in blood levels (within minutes) followed by rapid elimination (Hlozek et al., 2017). A comparison between vaporization and IP administration of THC illustrates that these routes produce different blood levels of THC and its metabolite (11-OH- Δ^9 -THC) (Manwell et al., 2014a). Cannabinoids exhibit similar pharmacokinetics in both sexes, as plasma THC levels following inhalation are not significantly different between male and female rats (Javadi-Paydar et al., 2018). In mice, concentrations of THC are similar in blood and brain following IV injection and aerosol INH at the antinociceptive ED₅₀ value (Wilson et al., 2002), suggesting that observed behavioral responses to cannabinoids reflect the amount of drug bioavailable in the CNS.

The most widely utilized preclinical model of cannabinoid behavior is the tetrad test (Martin et al., 1991), where rodents experience four distinct behavioral outcomes following drug exposure: hypomotility (reduced activity), hypothermia, analgesia, and catalepsy. Rodents injected with THC or other cannabinoids exhibit all four tetrad responses, which can be blocked by genetic (*e.g.*, CB₁ receptor knockout mice) or pharmacological (*e.g.*, CB₁

receptor antagonists) intervention. Following exposure to THC vapor using an ENDS device, rats display dose-dependent hypothermia and hypomotility (Nguyen et al., 2016b), and both hypothermic and analgesic effects of THC vapor are attenuated by pretreatment with a CB1 receptor antagonist. Many of the acute effects of THC inhalation appear to be consistent across both sexes as well as different ages (Javadi-Paydar et al., 2018; Nguyen et al., 2016b), with repeated exposures of vaporized THC inducing tolerance to hypothermia in both male and female rats (Nguyen et al., 2018). However, studies of chronic THC vapor exposure report sex-specific behavioral changes, with females but not males exhibiting signs of tolerance and increased rates of fentanyl IVSA (Nguyen et al., 2019a). By contrast in male rats, THC inhalation seems to reduce consumption of opioids by self-administration, suggesting a potential therapeutic effect of cannabinoids on opioid abuse in males (Nguyen et al., 2019b).

Among the new psychoactive substances, synthetic cannabinoids are indisputably the most popular among drug users (Miliano et al., 2018; Miliano et al., 2016), and the availability of ENDS and e-liquids containing these compounds has contributed in their popularity (Blundell et al., 2018). It has been demonstrated that first-generation synthetic cannabinoids (e.g. JWH-018, JWH-073, JWH-250) produce effects qualitatively similar to those of vaporized THC in three distinct tetrad behaviors (hypomotility, hypothermia, analgesia) in rats (De Luca et al., 2015) and mice (Ossato et al., 2016). None of these first-generation synthetic cannabinoids elicited the cataleptic effect produced by THC (De Luca et al., 2015; Ossato et al., 2016) yet it is possible that the dose tested may be insufficient to produce this response (Marshall et al., 2014). Highly potent second-generation synthetic cannabinoids (e.g. AB-CHMINACA, XLR-11) generate robust tetrad behavior following both IP and INH exposure (Lefever et al., 2017b). In accordance with the expected pharmacokinetics between these routes of administration, vapor exposure using ENDS results in a faster onset of effects and a shortened duration of action.

Beyond the tetrad test, cannabinoids produce changes in reward-like behavior and cognitive performance in rodents. Vaporized THC is more effective in stimulating feeding behavior and eliciting transient changes in locomotor activity (Hlozek et al., 2017; Manwell et al., 2014b). In addition, THC vaporization induces conditioned place preference (CPP) in contrast to place aversion occurring with IP administration (Manwell et al., 2014a), and does not lead to morphine cross-sensitization as others have observed with parenteral administration (Manwell et al., 2014b). Studies evaluating THC effects on learning and memory in mice demonstrate that Morris water maze performance is impaired in a dose-dependent manner following IP administration, while only high doses of inhaled marijuana achieve this response (Niyuhire et al., 2007). Likewise, inhalation of synthetic cannabinoids produces THC-like effects in a drug discrimination paradigm in both sexes of mice (Wiley et al., 2019). Unexpectedly, however, sexual dimorphism in drug potency following vapor exposure is observed for certain synthetic cannabinoids, suggesting that sex-based differences in pharmacokinetic parameters may influence preferences for specific compounds.

Stimulants:

The principal and most common route of administration for psychomotor stimulant drugs such as methamphetamine is via inhalation, which may exacerbate the risk of developing dependence (Das-Douglas et al., 2008; Heinzerling et al., 2010; Wood et al., 2008). Evaluation of methamphetamine inhalation in humans under controlled laboratory conditions that produce a subjective high reveals that in addition its psychoactive properties, this drug exerts cardiovascular effects (*i.e.*, changes in blood pressure, heart rate, myocardial contractility, stroke volume, cardiac output) that promote the development of long-term complications (Perez-Reyes et al., 1991). According to the SAMHSA/TEDS treatment admission database (SAMHSA, 2012), of those seeking treatment for drug dependence in the US, 4.7% were admitted for smoked cocaine vs 2.2% for other routes of cocaine administration. Indeed, the addictive liability of cocaine is increased significantly by usage of the “smokable” free base (crack cocaine), with more rapid absorption than via snorting (Allain et al., 2015). In addition, the emergence of e-cigarette technology has further expedited ingestion of methamphetamine (Rass et al., 2015), synthetic cathinones or ‘bath salts’ (Johnson and Johnson, 2014; Nguyen et al., 2016b), and the second-generation synthetic cathinone ‘flakka’ (α -pyrrolidinopentiophenone, α -PVP) (Aarde et al., 2015).

Substantial evidence indicates that acute stimulant inhalation in rodents produces similar biological effects to those that occur following more traditional routes of administration. Rats receiving different types of stimulants (methamphetamine, methcathinone, cocaine free base) via a nosecone nebulizer system exhibit a dose-dependent increase in locomotor activity, as is observed after IV and INH exposure (Meng et al., 1999). Likewise, biochemical analyses show that these drugs have equivalent bioavailability in brain and plasma. Using a commercially available ENDS vacuum system, acute exposure of rats to stimulants (methamphetamine, 3,4-methylenedioxypyrovalerone, mephedrone) decreases ICSS thresholds and enhances locomotor activity, with maximal effects similar to IP injection (Nguyen et al., 2016a). Furthermore, pretreatment of rats with the dopamine receptor antagonist SCH23390 blocked stimulant-induced increases in locomotor activity, suggesting that ENDS exposure produces drug concentrations that facilitate reward-like behavior. Similar findings have been reported following acute exposure in mice, where delivery of stimulants (methamphetamine, α -pyrrolidinovalerophenone) via IP injection or ENDS increases locomotor activity in a dose-dependent manner, with similar maximal effects for both routes of administration in males and females (Marusich et al., 2016). While the duration of action is extended in vapor-exposed mice compared with IP injection, ENDS exposure tends to elicit a smaller number of somatic signs of withdrawal (particularly in female mice). Repeated injections of either methamphetamine or α -pyrrolidinovalerophenone produce CPP, and there is a trend toward significance for α -pyrrolidinovalerophenone-induced locomotor sensitization after only 3 exposure sessions. Finally, exposing rats to caffeine by vapor inhalation produces drug-dependent increases in locomotor activity as well as dopamine release in the nucleus accumbens (Galvalisi et al., 2017). Collectively, these data support clinical findings demonstrating that ENDS can produce dose-dependent exposure to stimulants in rodents and may provide a useful technical approach for developing surgery-free self-administration paradigms for studying this class of drugs of abuse.

Opioids:

Illicit use of opioids has reached epidemic proportions in the United States, and in 2017 was declared a public health crisis (Volkow and Collins, 2017). After the release of crush-resistant oxycontin, there was a surge in usage of heroin followed by that of highly potent synthetic opioids such as fentanyl (Evans et al., 2019). This alarming trend, in combination with escalating abuse rates of prescription opioids, fueled the crisis (Dowell et al., 2016; Wilkerson et al., 2016). While opioids are unmatched in terms of efficacy for severe acute pain due to malignancy or terminal illness and are appropriate for short-term use in patients with chronic nonmalignant pain, concerns regarding potential risks of misuse, diversion and overdose mortality rates have limited their usage in patients (Busse et al., 2018; Finnerup et al., 2015). Initially, recreational opioid use occurs mainly via oral administration; however, intravenous injection or inhalation is practiced frequently by experienced users in order to capitalize on rapid and potent euphorogenic effects which enhance abuse liability (Gasior et al., 2016). In light of this epidemic, preclinical studies examining biological effects of intravenously administered or inhaled opioids are critical to advance our understanding of mechanisms underlying opioid addiction.

Intravenous self-administration represents the most common approach for evaluating rodents in preclinical models of addiction to opioids (*e.g.*, heroin, morphine, oxycodone, buprenorphine, and fentanyl); comparatively few rodent studies have evaluated opioid self-administration via inhalation. In mice, acute administration of some opioids by inhalation (heroin, fentanyl, meperidine) exhibits antinociceptive potencies similar to those obtained with IV exposure, whereas others (morphine, codeine) exhibit enhanced potency and CNS bioavailability (Lichtman et al., 1996). Studies using home-made nebulizer systems demonstrate that rats will self-administer aerosol generated from aqueous solutions of the opioid sufentanil ranging between 10 and 75 ug/ml in a dose-dependent manner (Jaffe et al., 1989) during 2 hour short-access (ShA) sessions. Operant responses in this paradigm appear to be goal-directed as responses are sustained by a fixed ratio 5 (FR5) schedule of reinforcement (Jaffe et al., 1989; Weinhold et al., 1993), and likewise diminished by pretreatment with the mu opioid receptor antagonist naloxone (Jaffe et al., 1989) or by the removal of sufentanil from the vapor solution (Weinhold et al., 1993). Recent work utilizing a commercially available ENDS vacuum system confirms that rats will self-administer vaporized e-liquid in a dose-dependent manner during 1 hour ShA sessions, with serum levels of sufentanil correlating to the number of vapor exposures (Vendruscolo et al., 2018). In addition, long-access (LgA) exposure during a 12-hour session produces significant escalation in operant responding for sufentanil over 13 sessions. While LgA rats develop enhanced mechanical hypersensitivity that emerges during between-session spontaneous abstinence, tactile thresholds are unchanged in SgA rats (Vendruscolo et al., 2018). Moreover, naloxone-precipitated withdrawal increases somatic signs of withdrawal in LgA rats. Collectively, these findings indicate that operant self-administration of sufentanil using ENDS exhibits both face and construct validity, and represents a useful method for investigating the neurobiological basis of opioid addiction.

Conclusions and Future Directions

Drug addiction is a chronic relapsing disorder driven by molecular changes that occur in the brain in response to habitual drug use, and currently available pharmacotherapies have shown only limited efficacy in reducing drug use and relapse in addicts. While ENDS technology is purported to curb cravings and to reduce harmful exposure to carcinogens and other toxic substances obtained from smoking, multiple preclinical lines of evidence suggest that usage of these delivery systems produces behavioral changes consistent with drug reward along with adverse effects on multiple organ systems. In light of these results, it is clear that caution should be exercised when considering the utilization of ENDS as medical devices. Future studies examining ENDS using well-controlled experimental conditions are critical in order to establish conclusively the potential deleterious health effects of exposure to both vehicle and active drug constituents from these devices via a route of administration pertinent for human use. The findings from such efforts would significantly enhance our understanding of mechanisms of drug reward, thereby validating the translational relevance of this paradigm and providing the foundation for discovery of druggable targets and novel therapeutics for treating addiction.

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Highlights

- Electronic nicotine delivery devices (ENDS) replacing tobacco use in youth populations
- Advances in ENDS technology facilitates preclinical inhalation exposure paradigms
- Chronic nicotine exposure by ENDS in rodents provide evidence of detrimental health effects
- ENDS evaluate addictive properties of other drugs of abuse in rodent via inhalation

Figure 1.

Prevalence of ENDS and rodent-based research. (A) Search interest for ENDS from 2009 to 2018 using Google Trends (searched 7/1/2019) with the terms “e-cigarette” and/or “vaping” (green), as well as recent nicotine-salt ENDS by JUUL Labs Inc. using the search term “JUUL” (blue). (B) Timeline of rodent ENDS publications to date, by year.

Figure 2. Search strategy for literature review. (A) Pubmed search strategy identified 90 relevant scientific publications describing the study of ENDS using rodent models. (B) Worldwide map of rodent ENDS publications to date, with countries indicated in green. For some countries (United States, Canada, Australia), individual states or provinces were highlighted for clarity.

Figure 3.
Schematics of the three major types of preclinical ENDS systems: (1) Aerosol Nebulizers, (2) Smoking Machines, and (3) Vacuum Exposure Systems.