

Running Head: Imaging the Effect of Electronic Cigarettes at  $\beta 2^*$ -nAChRs

**Use of Electronic Cigarettes Leads to Significant Beta2-Nicotinic Acetylcholine Receptor Occupancy: Evidence From a PET Imaging Study**

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## ABSTRACT

**Background:** Electronic cigarettes (ECs) can influence nicotine addiction by delivering aerosolized nicotine. We investigated if nicotine from ECs is delivered to the brain  $\beta_2^*$ -nicotinic-acetylcholine receptors ( $\beta_2^*$ -nAChR) and how this relates to the behavioral effects and nicotine delivery from cigarettes. **Methods:** Seven nicotine users participated in positron emission tomography (PET) studies with (-)-[ $^{18}\text{F}$ ]Flubatine before and after nicotine challenge with 0, 8 mg/ml, and 36 mg/ml nicotine in a 3.3 Volt, 1.5 Ohm EC or a standard tobacco cigarette. Craving was evaluated before and after product use. **Results:** Average  $\beta_2^*$ -nAChR occupancy was higher after 36 mg/ml EC challenge compared to 8 mg/ml EC at trend level. Average  $\beta_2^*$ -nAChR occupancy after tobacco cigarette smoking was  $68\pm 18\%$  and was not different compared with 8 mg/ml ( $64\pm 17\%$ ) or 36 mg/ml ( $84\pm 3\%$ ) nicotine in EC users. Area under the curve (AUC) of blood nicotine level was higher in the cigarette smoking group compared with the 8mg/ml group ( $p=0.03$ ), but similar compared with the 36 mg/ml EC ( $p=0.29$ ). Drug craving was reduced after use of the tobacco cigarette, 8 mg/ml EC, and 36 mg/ml EC.

**Conclusions:** In this novel investigation of EC effects at  $\beta_2^*$ -nAChRs, we show that average  $\beta_2^*$ -nAChR occupancy was higher after 36 mg/ml EC challenge compared with 8 mg/ml EC. Receptor occupancy and arterial blood nicotine levels after cigarette smoking were similar to 36 mg/ml EC use under controlled conditions. These findings suggest that the ECs studied here have abuse liability and may provide an adequate alternative nicotine delivery system for cigarette smokers.

## IMPLICATIONS

This is the first study to directly determine the neurologic effects of electronic cigarettes on human brain beta-2 nicotinic acetylcholine receptors using PET neuroimaging with (-)-

[ $^{18}\text{F}$ ]Flubatine, a novel radiotracer. Our findings suggest that the e-cigarettes studied here have abuse liability and may provide an adequate alternative nicotine delivery system for cigarette smokers.

## INTRODUCTION

Estimates suggest that tobacco cigarette smoking caused more than 20 million premature deaths since 1965 and is the most preventable cause of disease.<sup>1</sup> Although rates of dependence on nicotine, the primary addictive constituent of cigarette smoke, have stabilized at 15% for the general population in the U.S., nationwide smoking cessation rates continue to be low.<sup>2</sup>

Inhalation is the quickest way a drug can reach the brain and provide neurological effects.

Inhaled cigarette smoke delivers nicotine to the pulmonary circulation and reaches the arterial circulation and brain within seconds after inhalation, leading to a rapid neurologic effect and high abuse/dependence liability.<sup>3</sup> Nicotine replacement products designed to increase cessation rates have been developed in the form of patches, gum, lozenges, nasal spray, and inhaler.

However, they deliver nicotine more slowly compared to cigarette smoking and may not be as rewarding to smokers.<sup>4</sup> Recently, electronic cigarettes (EC) have gained popularity among experienced cigarette smokers as well as tobacco-naïve users.<sup>5</sup> However, scientific information about ECs to inform consumers has been limited due in part to rapidly evolving EC design features.

ECs are battery-operated devices that heat and aerosolize a liquid that typically contains propylene glycol, vegetable glycerin, flavorants, and the psychoactive drug nicotine. Although initial studies of early-model ECs suggested that their ability to deliver nicotine was minimal,<sup>6</sup> later studies with more advanced products found that some ECs can deliver nicotine to the venous blood in physiologically active doses and, in some cases, that EC-delivered nicotine is

comparable to conventional cigarettes.<sup>7-11</sup> Further, advances in product technology have led to the development of more sophisticated ECs (i.e. personal vaporizers) with larger batteries and tank systems that are capable of producing larger amounts of aerosol and greater nicotine delivery as compared to earlier EC products.<sup>7,10,12</sup> ECs have the potential to engender and perpetuate nicotine dependence in non-users and youth, but may also have the potential to reduce harm in cigarette smokers.<sup>13,14</sup> Identifying EC mechanisms of action in the brain, potential abuse liability, comparison to tobacco cigarettes, and the relationship between mechanism of action and user behavior are crucial steps in evaluating these products, understanding their effects, and proposing regulation that maximizes potential benefit while minimizing potential harm.

Nicotine binds with high affinity to the brain beta2 nicotinic acetylcholine receptors ( $\beta_2^*$ -nAChRs), which upregulate (increase in number) after chronic nicotine use<sup>15,16</sup>. Receptor imaging with single photon emission computed tomography (SPECT) or PET has been used successfully to examine mechanisms of nicotine dependence through evaluation of  $\beta_2^*$ -nAChR availability as a consequence of cigarette smoking<sup>4,17,18</sup> as well as occupancy by nicotine in multiple previous studies.<sup>16,19,20</sup> Findings from these studies suggest that cigarette smokers maintain near complete receptor occupancy throughout the day<sup>4,19,20</sup>, and that lower receptor occupancy levels lead to craving.<sup>4</sup> No previous studies have determined the degree to which ECs deliver nicotine to the human brain.

The primary aim of this study was to use PET imaging and (-)-[<sup>18</sup>F]Flubatine to evaluate the amount of nicotine delivered to brain  $\beta_2^*$ -nAChRs and arterial blood from EC users puffing from a single cartomizer-type EC (3.3 Volt; 1.5 Ohm) loaded with one of three different concentrations of nicotine liquid (0, 8, and 36 mg/ml). We hypothesized that nicotine delivery to brain and blood (as measured by  $\beta_2^*$ -nAChR occupancy and arterial nicotine concentrations)

would be greater in the higher active nicotine concentration liquid compared to the lower or zero concentration liquid. We also hypothesized that higher nicotine delivery would result in higher ratings of product liking and wanting, subjective responses that can reflect abuse liability.<sup>21</sup> As an exploratory aim, we compared receptor occupancy and arterial blood nicotine levels of the EC users to tobacco cigarette smokers.

## METHODS

### Participants

This study was approved by the Yale Institutional Review Board, Radioactive Drug Research Committee and by the Yale-New Haven Hospital Radiation Safety Committee. Four experienced EC users and 3 cigarette smokers participated in the study. After completing informed consent, subjects had a physical and neurological examination and an electrocardiogram (EKG). Lab tests were performed to exclude medical complications. Inclusion criteria were: (1) Men and women, aged 18-60 years, (2) Able to read and write English, (3) Able to give voluntary, written informed consent, (4) Daily EC use for **at least 1 month among** EC subjects or smoked >10 cig/day **for the past year** for cigarette smokers; (5) Urine cotinine levels > 50 ng/ml at intake; (6) CO levels > 7 ppm at intake for smokers; (7) non-treatment seeking. Exclusion criteria were: (1) Current medical condition such as neurological, cardiovascular, endocrine, renal, liver, or thyroid pathology; (2) History of or current neurological or psychiatric disorder including drug or alcohol dependence (as per DSM-IV) except nicotine dependence; (3) Regular or current use of any prescription, herbal or illegal psychotropic medications in the past 1 year, with no current illegal drug use confirmed by urine toxicology; (4) Drink more than 21 drinks per week for women or 35 drinks per week for men<sup>22</sup>; (5) Women who are pregnant or nursing; (6)

Individuals currently taking medication that may affect cholinergic system or nicotine replacement therapy prescribed for smoking cessation; (7) Contraindications to MRI such as claustrophobia or metal in their body.

Clinical Assessments: A standardized battery was administered to all subjects for diagnostic purposes at intake including the Structured Clinical Interview for DSM-IV Disorders (SCID),<sup>23</sup> and assessments of demographics, measures of tobacco use, alcohol and drug use, family history of smoking, mood (Beck Depression Inventory<sup>24</sup>, Center for Epidemiologic Studies Depression Scale<sup>25</sup>), anxiety (State Trait Anxiety Inventory<sup>26</sup>), impulsivity (Barratt Impulsivity Scale<sup>27</sup>), smoking dependence (FTND<sup>28</sup>), smoking/EC craving (Questionnaire on Smoking Urges Brief (QSU<sup>29</sup> subscales of intention/desire to smoke and urge for relief of negative affect<sup>30</sup> and adapted for ECs) and withdrawal (Nicotine Withdrawal questionnaire<sup>31</sup>). Product liking and the Drug Effects Questionnaire<sup>32</sup> were assessed on a subjective scale (0-100, low-high) immediately following product use.

Nicotine abstinence: All participants were helped to remain abstinent for 5 days prior to each PET scan with contingency management techniques that have been used extensively in our laboratory.<sup>16,17,33</sup> Specifically, we met with subjects on a daily basis over the 5 days of abstinence to provide support and obtain CO and urine cotinine levels to ensure smoking abstinence. Subjects were permitted to return to smoking/EC use as usual after each PET scan and repeat smoking cessation/EC abstinence prior to subsequent PET scans.

Imaging Procedures: Prior to the PET scans, a T1-weighted MRI was acquired for all subjects on a 3.0 Tesla Siemens Trio camera (Siemens, Erlangen, Germany) to rule out any brain abnormalities and for use in the delineation of regions of interest. A high resolution, three-

dimensional Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE) T1-weighted sequence was used to acquire sagittal images for anatomical determinations and co-registration (TR=1500ms, TE=2.83ms, FOV=256 x 256mm<sup>2</sup>, matrix=256 x 256mm<sup>2</sup>, slice thickness=1.0mm without gap, 160 slices, voxel size 1.0 x 1.0 x 1.0 mm<sup>3</sup>).

PET data were acquired on the Siemens High Resolution Research Tomograph (HRRT), the highest sensitivity and resolution human brain PET scanner.<sup>34</sup> A 6- minute transmission scan was acquired to correct for attenuation. List-mode data were reconstructed with OSEM with built-in corrections for attenuation, normalization, scatter, randoms, deadtime and subject motion.<sup>35,36</sup> The radiotracer (-)-[<sup>18</sup>F]Flubatine was synthesized<sup>37</sup> and administered on PET scan days as a bolus plus constant infusion with a  $K_{bol}$  of 360 min over 3.5 hours. The total injected [<sup>18</sup>F]Flubatine dose was not statistically different between subjects undergoing similar scans (0 mg/ml EC: 294±4 mBq; 8 mg/ml EC: 276±17 mBq; 36 mg/ml EC: 262±21 mBq; Tobacco Cigarette: 200±17 mBq (p = 0.076). Injected cold mass was not different between subjects undergoing similar scans (0 mg/ml EC: 0.10±0.089 µg; 8 mg/ml EC: 0.30±0.25 µg; 36 mg/ml EC: 0.17±0.17 µg); Tobacco Cigarette: 0.18±0.19 µg (p = 0.665). **Specific activity of [<sup>18</sup>F]Flubatine was: 0 mg/ml EC - 2028 ± 155 mBq/nmol; 8 mg/ml EC – 572 ± 572 mBq/nmol; 36 mg/ml EC – 1299 ± 881 mBq/nmol; Tobacco cigarette – 909 ± 688 mBq/nmol.** Arterial blood samples were taken to measure the metabolite-corrected parent [<sup>18</sup>F]flubatine input function as previously described<sup>38</sup> and nicotine concentration post challenge (t = 0, 0.3, 1, 3, 5, 10, 20, 30, 60, 90 minutes). Baseline receptor availability measure was obtained 90-120 minutes after radiotracer infusion as previously validated.<sup>39</sup> Subjects started a challenge [ECs (counterbalanced order) or tobacco cigarette] at 125 minutes for 5 minutes in the scanner. Post nicotine scanning continued until the end of 210 minutes of infusion. The time period used to

quantify occupancy was 180-210 minutes (55-85 minutes post challenge). **A representative time activity curve of [ $^{18}\text{F}$ ]Flubatine in an EC user is shown in Figure 1.** Subjects were given at least 2 weeks between each scan to allow for smoking or EC use resumption/cessation and arterial line healing. EC users participated in 2 scans (8 mg/ml and 36 mg/ml EC; N=2) or 3 scans (0, 8 and 36 mg/ml; N=2), while cigarette smokers participated in 1 scan (tobacco cigarette only).

Image Analysis and Outcome Measure: The primary outcome measure for this study was (-)-[ $^{18}\text{F}$ ]flubatine volume of distribution ( $V_T$ ), estimated using the equilibrium ratio between total radioactivity concentration in brain and parent (-)-[ $^{18}\text{F}$ ]flubatine concentration in arterial plasma. This is proportional to the number of  $\beta_2^*$ -nAChRs available for (-)-[ $^{18}\text{F}$ ]flubatine binding, thus we refer to this as ‘receptor availability’. Baseline receptor availability was measured 90-120 minutes after radiotracer infusion. Subjects started a challenge [ECs (counterbalanced order) or tobacco cigarette] at 125 minutes for 5 minutes in the scanner. The time period used to quantify occupancy was 180-210 minutes (55-85 minutes post challenge). Post nicotine scanning continued until the end of 210 min of infusion. Occupancy of  $\beta_2^*$ -nAChRs by nicotine was evaluated using the Lassen plot approach.<sup>40</sup> **The regions of interest used for calculation of receptor occupancy were: cerebellum, frontal, parietal, temporal, occipital, caudate, putamen, hippocampus, and amygdala. These regions were used because they were previously examined in  $\beta_2^*$ -nAChR studies.<sup>4,19,38,39</sup> Since there were no between-region differences in nicotine occupancy or physostigmine induced displacement by ACh,<sup>38,39</sup> we did not focus on any particular region and assumed uniform uptake of nicotine from the ECs and tobacco cigarettes in this study. The thalamus was not included because equilibrium was not reached in this region with the bolus-infusion paradigm. Lassen plots in representative subjects are in Figure 2.**



Use of EC: Based on the findings of recent studies that showed use of ECs led to similar venous plasma nicotine levels as cigarette smoking within 5 minutes of use<sup>7,9,11</sup>, we used an e-Go type EC battery (3.3 V, 1000 mAh) with 1.5 ohm dual-coil 510-style cartomizer and a 70/30 propylene glycol/vegetable glycerin e-liquid ("tobacco flavor") with nicotine concentration of 0 mg/ml (n=2), 8 mg/ml (n=4) and 36 mg/ml (n=4). **E-liquid nicotine concentrations were measured with a validated liquid chromatography tandem mass spectrometry (LC-MS/MS) method with a linear range of 0.5 to 50 mcg/mL. Initially, a 0.050 mL aliquot of each liquid was diluted 1000-fold in acetonitrile, along with the addition of a stable isotopically labeled internal standard (nicotine-d4). This sample was further processed and injected (0.010 mL) into the LC-MS for quantitative analysis. Quantification was determined with a linear, 1/X weighting regression model using peak area ratios (nicotine peak area/nicotine-d4 peak area) versus concentrations.**

Subjects were instructed to puff on the EC once every 30 seconds for 5 minutes (10 puffs total) in a manner as they normally would. **A training session to familiarize EC users with the EC product used in the study was conducted at an appointment prior to the PET scans. All users were directly observed during EC use to ensure they did not have any problems operating the device.**

Use of tobacco cigarettes: Cigarette smokers were provided with a standard cigarette (Camel, Turkish and Domestic blend) and instructed to take one puff every 30 seconds for 5 minutes (10 puffs total) in a manner as they normally would.

Blood Samples: On PET scan days, arterial blood samples were collected prior to radiotracer administration and at multiple time points after EC or cigarette use to determine nicotine and cotinine concentrations in the blood, which were assayed using liquid chromatography tandem-mass spectrometry with deuterated internal standards.<sup>41</sup>

### Data Analysis

Statistical analyses were conducted using SPSS version 24. All outcomes were summarized descriptively, and nonparametric analyses were performed for all group comparisons. For comparison between cigarette smokers and EC users, the Mann-Whitney U test for independent samples was performed. For within group comparisons, the Wilcoxon Signed Rank test for related samples was performed. All tests were two-sided and considered statistically significant at  $\alpha=0.05$ .

## **RESULTS**

**Participants** –Four healthy EC users participated in two scans each (8 mg/ml and 36 mg/ml EC), and two of the users underwent a third scan with a placebo (0 mg/ml EC). The mean age was  $26\pm 4$  years, and 1 subject was female. The baseline urine cotinine level was  $875\pm 144$  ng/ml, and the baseline exhaled carbon monoxide level was  $6\pm 1$  ppm. Three healthy smokers participated in one scan with cigarette challenge. The mean age was  $45\pm 16$  years, and 1 subject was female. The baseline urine cotinine level was  $783\pm 385$  ng/ml, **FTND was  $5.3\pm 2.5$**  and the baseline exhaled carbon monoxide level was  $30\pm 28$  ppm.

Occupancy and Arterial Blood Nicotine Levels Following EC Use

**$\beta_2^*$ -nAChR occupancy by nicotine** –  $\beta_2^*$ -nAChR occupancy is summarized in Table 1, and  $V_T$  images from a subject who participated in three separate scans at different EC liquid nicotine concentrations (0, 8, and 36 mg/ml) are shown in Figure 3. The average  $\beta_2^*$ -nAChR occupancy was higher after 36 mg/ml EC challenge compared with 8 mg/ml EC at trend level ( $84 \pm 3\%$  vs.  $64 \pm 17\%$ ,  $p = 0.07$ ). The occupancy range was 41.6-81.9% after 8 mg/ml EC compared with 80.2-88.8% after 36 mg/ml EC. Two of the users achieved 70% occupancy following exposure to the 8 mg/ml EC condition. The use of the 0 mg/ml nicotine EC did not lead to significant receptor occupancy by nicotine ( $2.5 \pm 5.5\%$ ).

**Arterial blood plasma nicotine levels** –  $C_{max}$  and AUC from 0-90 minutes were higher after 36 mg/ml EC compared to 8 mg/ml EC at trend level ( $12 \pm 5$  ng/ml,  $389 \pm 137$  ng\*min/ml; and  $6 \pm 4$  ng/ml,  $175 \pm 146$  ng\*min/ml, respectively;  $p = 0.07$ ; Table 1 & Figure 4). No nicotine was detected in the plasma in the 0 mg/ml condition.  $T_{max}$  was not different following use of different ECs (8 mg/ml EC:  $4.5 \pm 1$  min; 36 mg/ml EC:  $5 \pm 0$  min).

**Liking and Craving Ratings** – Ratings of product liking were similar after each EC use (0 mg/ml =  $80 \pm 28$ ; 8 mg/ml =  $75 \pm 38$ ; 36 mg/ml =  $74 \pm 26$ ). Craving was reduced at trend level as measured by the QSU brief scores<sup>30</sup> before and after use of 8 mg/ml EC [desire ( $13 \pm 1$  vs.  $9 \pm 3$ ;  $p = 0.07$ ) and the 36 mg/ml EC [desire ( $11 \pm 3$  vs.  $8 \pm 3$ ;  $p = 0.11$ ), relief ( $11 \pm 6$  vs.  $9 \pm 5$ ;  $p = 0.11$ ).

Desire and relief were not significantly lower after the 0 mg/ml EC.

The change in desire and relief following challenge with the 8 mg/ml EC compared with the 36 mg/ml EC was not significantly different ( $p = 0.29$ ). Subjective drug effects were similar across conditions.

Exploratory Comparison of Occupancy and Arterial Blood Nicotine Levels Following EC and Cigarette Use

**Receptor occupancy by nicotine** – The average  $\beta_2^*$ -nAChR occupancy after the smoking challenge was  $68.4 \pm 18\%$  (range 52.9-88.9%) and was not significantly different compared with the 8 mg/ml or 36 mg/ml EC ( $p=0.48$ ).

**Arterial blood plasma nicotine levels** –  $C_{\max}$  following cigarette smoking was ( $27 \pm 2$  ng/ml), which was significantly higher than the  $C_{\max}$  following EC use during both 8 and 36 mg/ml conditions ( $6 \pm 4$  ng/ml, vs.  $12 \pm 5$  ng/ml;  $p = 0.03$ ). AUC in the cigarette smoking group was  $516 \pm 101$  ng\*min/ml which was significantly higher compared AUC to the 8 mg/ml EC ( $p=0.03$ ) but not compared with 36 mg/ml EC ( $p=0.29$ ) in the EC group.  $T_{\max}$  was 5 minutes for all trials (not different compared to EC use).

**Liking and Craving Ratings** – Liking following use of the tobacco cigarette was [ $37 \pm 40$ ]; this did not differ compared with the EC at either liquid strength (8 mg/ml:  $75 \pm 38$ ; 36 mg/ml:  $74 \pm 26$ ). Craving was lower, but not significantly, before and after cigarette smoking [desire ( $8 \pm 3$  vs.  $5 \pm 5$ ;  $p = 0.20$ ; relief ( $5 \pm 2$  vs.  $3 \pm 0$ ;  $p = 0.20$ )]. The change in score was not different after cigarette smoking compared with 8 mg/ml or 36 mg/ml nicotine solutions by the EC group.

## DISCUSSION

This study is the first to examine the in-vivo occupancy of nicotine at brain  $\beta_2^*$ -nAChRs in healthy EC users following exposure to three different nicotine concentrations (0, 8, and 36 mg/ml) administered using a 3.3 Volt, 1.5 Ohm EC. Preliminary results suggest that, under the

conditions reported here, EC users can achieve brain  $\beta_2^*$ -nAChR occupancy similar to that of regular cigarette smokers, although nicotine levels in plasma tend to be lower. Furthermore, craving was reduced to a similar degree by use of ECs studied here and a tobacco cigarette.

#### Effects on $\beta_2^*$ -nAChR Occupancy

The receptor occupancy levels in the cigarette smokers in our study ranged between 53-89% and were similar to levels noted previously.<sup>4,16,19,20</sup> **The smokers with lower receptor occupancy (subjects 6 and 7) were likely underestimated since one of them had a small amount of nicotine in the blood prior to scan and the other received a lower dose of injected radioactivity.** The finding that experienced EC users who had used an EC with 8 mg/ml or 36 mg/ml liquid nicotine were capable of reaching receptor occupancy values similar to tobacco cigarette use provides further evidence of the abuse liability of ECs. Among the EC users, we note that there was greater consistency in receptor occupancy after use of the higher concentration nicotine liquid with all users ranging between 80-89%. There was more variability in receptor occupancy after use of the lower concentration nicotine liquid, but importantly, two of the subjects reached occupancy of 70% and 81% with this concentration. This finding indicates that, in the device reported here, use of 8 mg/ml nicotine is capable of providing tobacco smoke-like receptor occupancy, but that like cigarette smoking, user behavior is an important determinant of nicotine intake. Moreover, recent results from other laboratories suggest that even lower strength liquids (e.g., 4 mg/ml) might be capable of similar effects, at least when paired with more powerful EC devices (e.g., 4 Volt, 0.4 Ohms).<sup>12</sup>

### Effects on Arterial Blood Nicotine Uptake

Cigarette smokers exhibited higher arterial blood nicotine  $C_{\max}$  with less variability than EC users, which is consistent with prior studies suggesting that cigarette smoke delivers nicotine more efficiently than some ECs as measured by venous blood concentrations<sup>42</sup>, but inconsistent with other studies that showed similar uptake patterns.<sup>9,11,43</sup> However,  $T_{\max}$  and AUC were similar after cigarette use compared to 36 mg/ml EC use. These findings suggest similar brain nicotine delivery between these two products. We note, however, that puffing conditions were strictly controlled. The level of  $C_{\max}$  and AUC individuals would have reached if they had puffed ad lib or over a period of time longer than 5 minutes is unclear. Among cigarette smokers, the average  $C_{\max}$  was lower than that previously observed<sup>4</sup>, likely due to the limited duration and number of puffs permitted during the experiment. Whether these findings would remain consistent after ad lib (i.e. real-world) use of the two products or in naïve users remains to be determined. Given the similarities between the products noted, the results suggest that the 36 mg/ml EC used here has the potential to perpetuate nicotine addiction but also to provide an effective nicotine delivery system for cigarette smokers.

When comparing within subject use of higher versus lower concentration liquid, there was a trend toward higher  $C_{\max}$  and AUC after use of the high dose EC. Notably, even the lower concentration liquid was capable of delivering nicotine efficiently in some of these users. This result may suggest compensatory behavior (i.e. taking larger and/or longer puffs from the EC when lower concentration liquid was in it) in order to titrate to a desired nicotine level. The compensation phenomenon has been noted recently in EC users<sup>44</sup> and has been documented extensively in cigarette smokers.<sup>45</sup> Future studies with EC users may benefit from concurrent measurement of puff topography in order to explore this possibility.<sup>10</sup>

### Effects on Product Liking/Craving

We hypothesized that liking would increase with higher nicotine strength liquids; however, we found no significant differences in product liking or wanting despite trends toward higher nicotine uptake following use of an EC with higher nicotine concentration liquid. Craving was also reduced to a similar degree across groups. There are several potential explanations for these findings. First, recent work has observed that perceived harshness of ECs increases with increasing nicotine strength and that menthol attenuates this effect.<sup>46</sup> Since we used a non-menthol “tobacco” flavored liquid, any potential increase in nicotine delivery may have been accompanied by an increase in harshness, which in turn could have attenuated liking and craving reduction. Future studies may consider the role of menthol and flavors in modifying product desirability, and particularly whether flavorants added to the liquid increase the appeal of liquids with higher nicotine concentrations. Second, other product characteristics may play a significant role in determining product acceptability. EC users were not using their preferred device and liquid, while cigarette smokers were not using their preferred cigarette brand. Since all users were using an unfamiliar product, it is probable that the differences in product liking and drug effects did not contribute significantly to the findings.

### Study Limitations

This study has several limitations. First, only healthy EC users were enrolled and they were younger than the exploratory comparison group of cigarette smokers. The EC users were young and may not be as highly nicotine dependent as other populations. We therefore cannot generalize our findings to non-users who may initiate EC use, persons with medical and/or

psychiatric comorbidities, more highly nicotine dependent persons, and older users. Second, the sample size was small. Because this study is preliminary, there remains the possibility that we were underpowered to detect differences between nicotine solution strengths with respect to liking and drug effects. Replication of this study with a larger sample size is necessary in order to validate the observed physiologic effects of EC use. Third, subjects puffed the ECs on a fixed schedule of one puff every 30 seconds for 5 minutes. It is possible that receptor occupancy, arterial blood, and subjective measures of product liking may have varied if subjects were permitted to puff ad-lib. Finally, we used a single EC device and a specific liquid formulation in a single flavor. ECs are a heterogeneous group of products that differ considerably across products in key factors such as battery voltage, heater resistance, materials, and construction. Moreover, the nicotine liquids are also extremely variable, differing by propylene glycol/vegetable glycerin ratio, nicotine concentration, and flavorants added. Similarly, a standard tobacco cigarette was used that may not reflect the product characteristics to which users were previously accustomed. This could have affected all the biologic and subjective drug effects that were measured.

#### Future Directions

Another important question that remains (that could not be answered by this study) is whether equivalent nicotine receptor occupancy necessarily suggests equivalent abuse liability when comparing ECs to combustible tobacco products. While nicotine is the primary addictive component of tobacco, smoke contains more than 7,000 chemicals when burned and is a much more complex mixture than EC aerosol.<sup>47</sup> Also, smoke constituents such as aldehyde products can potentiate the effects of nicotine by acting as MAO-I and reducing the metabolism of



dopamine.<sup>48</sup> Future imaging studies examining these differences may provide important insights as to whether ECs are capable of equivalent dopamine release as compared to tobacco cigarettes.

## CONCLUSIONS

The average  $\beta_2^*$ -nAChR occupancy was higher after 36 mg/ml EC challenge compared with 8 mg/ml EC at trend level, but evidence of compensatory behavior was noted in two users under the 8 mg/ml condition. Cigarette smokers and EC users had similar  $\beta_2^*$ -nAChR occupancy,  $T_{max}$ , and reductions in craving, while smokers reached higher maximal arterial blood nicotine concentrations after puffing under controlled conditions. AUC did not differ between tobacco smoking and use of the 36 mg/ml EC, indicating similar nicotine delivery to the brain and arterial blood. These findings suggest that the ECs studied here have abuse liability, but may also provide an effective nicotine delivery system for cigarette smokers. Undoubtedly additional studies are required with a larger sample size and in different participant populations to expand our understanding of EC abuse liability and how they compare to traditional cigarettes.

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**Declaration of Interests.** Dr. Eissenberg is listed as a co-inventor on a pending patent describing a device intended to measure puffing behavior in users of a variety of electronic cigarette products. There are no other relevant conflicts of interest.

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TABLE 1:  $\beta_2^*$ -nAChR occupancy, Maximum Arterial Blood Nicotine Concentration, and Area Under the Curve After Use of 8 mg/ml EC, 36 mg/ml EC, and Tobacco Cigarette

Subject No.	% Receptor Occupancy after 8 mg/ml EC	% Receptor Occupancy after 36 mg/ml EC	% Receptor Occupancy after TOBACCO cigarette	$V_{ND}$ average (ml/cm <sup>3</sup> )	$C_{max}$ (ng/ml) after 8 mg/ml EC	$C_{max}$ (ng/ml) after 36 mg/ml EC	$C_{max}$ (ng/ml) after TOBACCO cigarette	$T_{max}$ (min) after 8 mg/ml EC	$T_{max}$ (min) after 36 mg/ml EC	$T_{max}$ (min) after TOBACCO cigarette	AUC after 8 mg/ml EC (ng*min/ml)	AUC after 36 mg/ml EC (ng*min/ml)	AUC after TOBACCO cigarette (ng*min/ml)	Average [NIC] 0-90 min after 8 mg/ml EC	Average [NIC] 0-90 min after 36 mg/ml EC	Average [NIC] 0-90 min after TOBACCO cigarette
1	41.6	83.6	N/A	6.00	2.9	17	N/A	3	5	N/A	123.7	457.9	N/A	0	2.7	N/A
2	64.3	80.2	N/A	6.30	4.2	5.9	N/A	5	5	N/A	61.5	224.4	N/A	0	1.9	N/A
3	80.9	88.6	N/A	6.27	11.1	12.5	N/A	5	5	N/A	389.5	537.0	N/A	2.7	3.7	N/A
4	70.2	84.0	N/A	6.77	5.9	13.1	N/A	5	5	N/A	126	336.0	N/A	0.5	2.1	N/A
5	N/A	N/A	88.9	6.79	N/A	N/A	29.3	N/A	N/A	5	N/A	N/A	625.2	N/A	N/A	4.0
6	N/A	N/A	52.9	5.67	N/A	N/A	25.3	N/A	N/A	5	N/A	N/A	498.3	N/A	N/A	2.7
7	N/A	N/A	63.3	7.43	N/A	N/A	26.7	N/A	N/A	5	N/A	N/A	425.3	N/A	N/A	2.1
<b>AVERAGE</b>	<b>64.3±17</b>	<b>84.1±3</b>	<b>68.4±18</b>	<b>6.46±0.58</b>	<b>6±4</b>	<b>12±5</b>	<b>27±2</b>	<b>4.5±1</b>	<b>5±0</b>	<b>5±0</b>	<b>175±146</b>	<b>389±137</b>	<b>516±101</b>	<b>1.2±1.7</b>	<b>2.2±1.4</b>	<b>2.9±0.97</b>

**Figure 1.** Time activity curve of [18F]Flubatine in a representative EC user. 55-85 minutes post-nicotine challenge was sufficient to reach a new steady state and allow calculation of receptor occupancy.



