A study of pyrazines in cigarettes and how additives might be used to enhance tobacco addiction

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Received 30 July 2014
Revised 31 March 2015
Accepted 1 April 2015
Published Online First
10 June 2015

ABSTRACT

Background Nicotine is known as the drug that is responsible for the addicted behaviour of tobacco users, but it has poor reinforcing effects when administered alone. Tobacco product design features enhance abuse liability by (A) optimising the dynamic delivery of nicotine to central nervous system receptors, and affecting smokers' withdrawal symptoms, mood and behaviour; and (B) effecting conditioned learning, through sensory cues, including aroma, touch and visual stimulation, to create perceptions of pending nicotine reward. This study examines the use of additives called 'pyrazines', which may enhance abuse potential, their introduction in 'lights' and subsequently in the highly market successful Marlboro Lights (Gold) cigarettes and eventually many major brands.

Methods We conducted internal tobacco industry research using online databases in conjunction with published scientific literature research, based on an iterative feedback process.

Results Tobacco manufacturers developed the use of a range of compounds, including pyrazines, in order to enhance 'light' cigarette products' acceptance and sales. Pyrazines with chemosensory and pharmacological effects were incorporated in the first 'full-flavour, low-tar' product achieving high market success. Such additives may enhance dependence by helping to optimise nicotine delivery and dosing and through cueing and learned behaviour.

Conclusions Cigarette additives and ingredients with chemosensory effects that promote addiction by acting synergistically with nicotine, increasing product appeal, easing smoking initiation, discouraging cessation or promoting relapse should be regulated by the US Food and Drug Administration. Current models of tobacco abuse liability could be revised to include more explicit roles with regard to non-nicotine constituents that enhance abuse potential.

To cite: Alpert HR, Agaku IT, Connolly GN. *Tob Control* 2016;**25**:444–450.

INTRODUCTION

Tobacco dependence is understood to be a complex process that is primarily caused by the pharmacological effects of nicotine which activate nicotinic acetylcholine receptors in the brain leading to release of the neurotransmitter dopamine into the mesolimbic area, corpus striatum and frontal cortex.[1–6] Dopamine release induces rewards, including pleasure, arousal, mental acuity and modulation of mood.[1] Since the 1980s, nicotine is believed to play a central role in biological reinforcement, tolerance and physical dependence, and withdrawal symptoms on discontinuation of intake.[7] However, substantial evidence exists to suggest that nicotine's reinforcing effects alone are not sufficient to account for the intense addictive properties of tobacco smoking and the high relapse rates among smokers after quitting even when provided nicotine in forms other than tobacco.[8–16] Further evidence that tobacco dependence entails more than addiction to nicotine includes the drug's limited ability to induce self-administration in animals;[17 18] lack of positive mood effects of pure nicotine in abstinent smokers;[19–21] lack of findings that nicotine in any other form than tobacco was preferred to placebo in normal smokers;[22 23] de-nicotinised cigarettes were as effective as regular cigarettes, and more than nicotine in any other delivery mode, in relieving withdrawal and craving;[24–27] and essential role of non-nicotine factors in cigarette addiction.[24 28]

The release of tobacco industry documents in the 1990s and investigation by the US Food and Drug Administration (FDA) brought to light tobacco manufacturers' research and development of the use of additives and ingredients besides nicotine which led to the increased appeal, attractiveness and addictiveness of products.[3–5 29] Independent scientific evidence has demonstrated that conditioned cues produced by tobacco non-nicotine ingredients and smoke constituents are instrumental in maintaining tobacco use.[6 7 16 22 28 30–35] Therefore, current models of tobacco product abuse potential recognise nicotine as the primary drug of addiction, and that non-nicotine tobacco constituents and sensory stimuli from packaging and environmental cues also contribute to tobacco dependence.[36]

Two major determinants of abuse potential are (A) dynamic pharmacokinetics of nicotine delivery and (B) learned behaviour effects triggered by sensory cues associated with use.[36 37] A smoker may feel the need to puff in order to attain threshold doses of nicotine and elicit the hedonic effects attributable to dopaminergic system reward pathways.[38] Nicotine delivery and its perception may be related to ease of the drug's administration and the 'impact' of tobacco smoke on posterior pharynx nociceptors, which is proposed to occur primarily by free nicotine.[39 40] Puff volume, speed of delivery, lung deposition, frequency of dosing, arterial absorption and other parameters affect the efficiency of nicotine delivery.[41]

Tobacco manufacturers modified the design of products by directly adding constituents to cigarettes that stimulate gustatory, tactile and olfactory nerve receptors and create chemosensory effects that could enhance elasticity in nicotine dosing as well as strengthen sensory cueing to optimise the 'pleasure' in smoking.[38 42–51] Pyrazines, a class of chemosensory agents, comprise 15 of the 599 compounds on the list of cigarette ingredients

provided by manufacturers to the US Department of Health and Human Services in 1994,[52] 8 of the compounds on the list of additive ingredients provided by manufacturers to the FDA in 2011[53] and 10 of the compounds presently listed on cigarette manufacturers' website as cigarette ingredients"[54–56] (box 1). The present study explores tobacco industry research that first identified pyrazines in tobacco smoke and was followed by the introduction of pyrazines in 'light' cigarettes and subsequent incorporation into Marlboro Lights and eventually in many other cigarette brands. It further examines their possible role in abuse potential.

METHODS

More than 7 000 000 tobacco industry documents have been disclosed by the major tobacco companies during litigation processes and made public as a result of the Minnesota Tobacco Trial and the Master Settlement Agreement of 1998.[57 58] We searched online internal tobacco industry document databases housed at Tobacco Documents Online (http://www.tobaccodocuments.org), the British American Tobacco Document Archive (http://bat.library.ucsf.edu) and the Legacy Tobacco Documents Library (http://legacy.library.ucsf.edu). Standard methods used for document analysis have been described in detail elsewhere.[59 60] Document identification was performed using an index-based word search of titles, authors, recipients and other document characteristics (such as date, document type, original file location), as well as keywords and abstracts. Whenever available, full-text optical character recognition was also used.

We used a snowball sampling method to first search the databases using an initial set of key words (eg, pyrazines, flavorant, flavoring, flavor, chemosensory, sensory, low-tar, stimulation, attributes, perception, effects, taste, smoothness and product development) and relevant combinations of these terms, and to generate further search terms from the documents identified. Relevant documents were abstracted and indexed. The resulting document set was surveyed for recurring authors, keywords, codes or project names that would suggest further avenues for retrieval.

A number of unique difficulties associated with the use of internal industry documents as a source of scientific information must be considered. Industry research was not generally subjected to careful peer review, and details regarding the experimental methods used and the resulting quality of the data are often unavailable, making it difficult to assess the reliability of the science. In addition, the available documents do not always represent the totality of the internal research that was conducted on a particular topic—as indicated by the existence of many partial reports and memos. Finally, within each given company, the documents are authored by numerous different researchers from a range of departments over tens of years, and so findings are sometimes inconsistent and occasionally even contradictory. Comparisons of the documents reveal real company-to-company differences in approach to the engineering of tobacco product design, a finding that must be taken into account. For these reasons and to inform the findings in internal industry documents, we conducted this research in conjunction with a systematic review of evidence from the current scientific literature indexed in databases including PubMed (http://www.pubmed.gov) and Web of Science (http://thomsonreuters.com/web-of-science) using the same search strategies.

Box 1 Pyrazine compounds in manufacturers' reports of cigarette ingredients

2-acetyl-3-ethylpyrazine,[52]
Acetylpyrazine,[52 53 55 56]
2,3-diethylpyrazine,[52–55]
2,3-dimethylpyrazine,[52]
2,5-dimethylpyrazine,[52–55]
2,6-dimethylpyrazine,[52]
2-ethyl(or methyl)-(3,s and 6)-methoxypyrazine,[52]
2-ethyl-3,(s or 6)-dimethylpyrazine,[52 53 55 56]
2-ethyl-3-methylpyrazine,[52 53 55]
2-isobutyl-3-methoxypyrazine,[52]
Methoxypyrazine,[52]
2-methylpyrazine,[52 53 55 56]
(Methylthio)methylpyrazine,[52]
2,3,5,6-tetramethylpyrazine,[52–56]
2,3,5-trimethylpyrazine,[52–56]
Methoxymethylpyrazine,[52 54 56]
[52]Manufacturers' 1994 cigarette ingredients report,
[53]Manufacturers' 2011 report to the Food and Drug Administration (FDA),
[54]Philip Morris, Inc. web site,
[55]Lorillard, Inc. web site,
[56]R.J. Reynolds Tobacco Company web site.

RESULTS

Introduction of pyrazines in cigarettes

The first US Surgeon General Report in 1964, which greatly increased concerns about the dangers of smoking, and the decline in cigarette sales beginning for the first time since World War II gave a major impetus to the tobacco industry's efforts to increase product appeal.[61] Tobacco manufacturers introduced new cigarette brands in response to these concerns, using filter ventilation, which lowered tar and nicotine yields or altered ratios measured under a standardised machine-based testing protocol.[38] The 'low-tar' cigarettes were found to have diminished taste, aroma and flavour and a weaker impact on receptors in the throat.[62 63] Facing a decrease in smoking and continuing 2% annual decline in cigarette sales, Philip Morris (PM) endeavoured to develop cigarettes with even lower tar yields, yet with taste and flavour that would satisfy smokers' 'palates and needs'.[62]

PM achieved a major breakthrough in this area by developing a 'full-flavour, low-tar product', marketed under the MERIT brand, which was the first 'light' cigarette.[62] The company accomplished this by first selecting out components of the volatile fraction of the particulate phase that contributed the greatest odour intensity from among the multitudes of aromatic chemicals and substances in tobacco smoke.[62] The gas chromatographic fractions of approximately 100 distinctive tobacco smoke flavourants were selected on the basis of high odour intensity as perceived by human participants using vapour dilution olfactometry.[62] The molecular structures of these compounds were then tentatively identified by high resolution mass spectrometry and by comparing the 'cracking', a term used by PM for fragmentation patterns, with known reference spectra. PM then incorporated the flavourants of highest intensity into the variety of compounds to be added to the reconstituted tobacco sheet. The reformulated cigarette flavour systems provided the taste, flavour and aroma qualities of the low-tar delivery cigarettes.[62] Finally, the company used panels of trained

flavour experts to evaluate the smoke flavour of prototypes. PM's research and development resulted in a cigarette yielding less than 9 mg tar with a smoke flavour of much higher tar yielding products.[62]

An extensive consumer testing programme of the new MERIT product was conducted, including blind interviews with nearly 3000 smoker panellists.[62 64] The majority of consumer participants reported that the new MERIT was equal or superior in taste to brands that delivered 60% more tar. Advertisements touted the product's 'enriched flavor' and described, "After twelve years of intensive research, Philip Morris scientists isolated certain key ingredients in smoke that deliver taste way out of proportion to tar." [65] (figure 1). This brand went on to capture a significant share of the low-tar cigarette market following its national launch in 1976.[62]

PM called the new flavour formulation 'Super Juice', which contained 2,6-dimethyl pyrazine, tetramethyl pyrazine and trimethyl pyrazine as well as acetic acid, cyclotene, maltol, isobutyric acid and 1-methyl indole.[66] Reverse engineering and research by British American Tobacco of PM products, MERIT, MERIT Menthol, Marlboro and Marlboro Lights identified at least six pyrazines: 2-methylpyrazine, methylethylpyrazine, dimethylethylpyrazine, 2,3-dimethylpyrazine, 2,6-dimethylpyrazine, trimethylpyrazine and tetramethylpyrazine, and found that pyrazines contribute to the burley flavour, which is a common characteristic of many PM brands (isomers noted only where referenced in document).[67] In the USA in the late 1970s, 'Super Juice'-like compounds were added to Marlboro Lights, which is now called 'Marlboro Gold'. Ingredients also included essential oils, inorganic acids and other constituents, added to a reconstituted tobacco sheet with diammonium phosphate, which appears to have allowed better control of constituent release.[68] Marlboro Lights have since become the leading selling cigarette brand.

Pyrazine flavour profiles

Pyrazines are heterocyclic aromatic organic compounds with the underlying chemical formula $C_4H_4N_2$. They are formed under pyrolytic conditions (temperatures ≥100°C) via the Maillard Browning reaction between amines and carbonyl compounds (generally sugars)[69 70] (figure 2), which occurs during the curing of tobacco leaf and during the smoking process.[71 72] Numerous pyrazine compounds have been detected in foods, which arise from the common practice of heating and Maillard


Cigarette Market Bombshell.

New 'Enriched Flavor' discovery for 9 mg. tar MERIT achieves taste of cigarettes having 60% more tar.

"Low tar, good taste."
Others have made the claim. Philip Morris just made the cigarette.
MERIT. Only 9 mg. tar. One of the lowest tar levels in smoking today.
Yet MERIT delivers extraordinary flavor. Flavor normally found only in higher tar cigarettes.
If you smoke, you'll be interested.

'Enriched Flavor' Boosts Taste—Not Tar

After twelve years of intensive research, Philip Morris scientists isolated certain key ingredients in smoke that deliver *taste way out of proportion to tar.*
The discovery's called 'Enriched Flavor.' It's extra flavor. Natural flavor. Flavor that can't burn out, can't fade out, can't do anything but come through for you.
We packed 'Enriched Flavor' into MERIT and began a series of taste tests.
The results were startling.

Smokers Report MERIT Delivers More Taste

9 mg. tar MERIT was taste-tested against five current leading low tar cigarette brands ranging from 11 mg. to 15 mg. tar.
Thousands of filter smokers were involved, smokers like yourself, all tested at home.
The results were conclusive:
Even if the cigarette tested had 60% more tar than MERIT, a significant majority of all smokers reported new 'Enriched Flavor' MERIT delivered more taste.
Repeat: delivered more taste.
In similar tests against 11 mg. to 15 mg. menthol brands, 9 mg. tar MERIT MENTHOL performed strongly too, delivering as much—*or more*—taste than the higher tar brands tested.
You've been smoking "low tar, good taste" claims long enough.
Now smoke the cigarette.
MERIT. Unprecedented flavor at 9 mg. tar.

MERIT and MERIT MENTHOL

*American Institute of Consumer Opinion. Study available free on request. Philip Morris Inc., Richmond, Va. 23261.

9 mg. "tar," 0.7 mg. nicotine av. per cigarette by FTC Method.

Warning: The Surgeon General Has Determined That Cigarette Smoking Is Dangerous to Your Health.

FAMILY CIRCLE 7/76 25

Figure 1 Advertisement for new 'Enriched Flavor' MERIT cigarettes (1976).

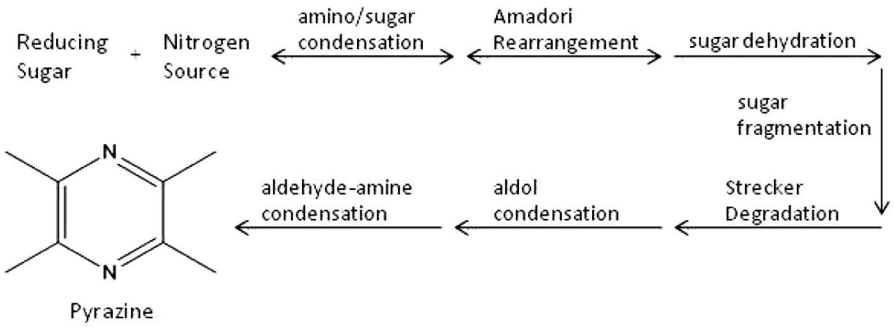

Figure 2 Graphic representation of the Maillard Browning reaction in the formation of pyrazines.

Pyrazines are yielded under pyrolytic conditions, mostly via the Maillard decomposition of Amadori compounds at temperatures ≥100°C. Shown is the tetramethyl version.

Browning reaction of sugars with protein and ammonia, providing a distinctive flavour. Other pyrazine compounds have been synthesised and promoted as flavouring agents because of their unique organoleptic properties and flavour and aroma profiles.[73–77] Pyrazines are 1 of 18 chemical classes of flavouring materials used in combustible tobacco products as described by Leffingwell *et al*.[78] They have been said to be among the most important compounds characterising the aroma and flavour of tobacco and tobacco smoke, contributing the 'brown notes' in general, and at least in some cases the cocoa, nutty or popcorn-type flavour notes.[79]

Chemosensory effects

Pyrazines are known to act on chemoreceptors, sensory receptors that transduce chemical signals into *action potentials*.[80] In addition to conveying the classical senses of taste and smell in humans, the mouth, nose and airways also contain chemosensory nerve endings of the trigeminal nerve.[81] These can be activated by physical stimuli as well as by a large array of chemical agents, leading to sensations such as burning, cooling and tingling, and contributing to flavour even in the absence of an olfactory percept.[82] Chemosensory effects of some other additives to cigarettes have been described, including essential oils (eg, menthol)[83] and organic acids (eg, levulinic acid).[84]

A report by the Tobacco Product Scientific Advisory Committee to the FDA described menthol's actions on transient receptor potential (TRP) channels, in particular TRPM8, which produce cooling and analgesia at low doses, irritation and pain at high doses, and desensitisation of the receptors with prolonged stimulation.[85] The report described how the addition of menthol in cigarettes creates perceptions of smoothness at low levels and analgesia at high levels and reduces the discomfort of smoking in long-term users. Results of population studies cited in the report showed youth being more likely to initiate smoking with a low menthol brand (eg, Newport), and older adults being less likely with a high menthol brand (eg, Kool).[85] An earlier review of internal tobacco industry documents reported the addition of levulinic acid to cigarettes to increase nicotine yields while enhancing perceptions of smoothness and mildness.[84 86]

Important chemosensory effects of pyrazines identified by the industry include smoothing, which may enhance the ease of inhalation and nicotine deposition by reducing the harshness and irritating effects of nicotine and other tobacco smoke constituents in the airways.[87] PM's internal documents of 1990 pertaining to the company's chemical senses research programme describe how a "chain of events from stimulation in the mouth, the throat and at the olfactory level leads to transmembrane electrical signals which are integrated in the brain."[88] According to these documents, diffusion and binding of constituents to receptors at sites of action, generation of action potentials, transmembrane signalling and integration of the diverse stimulus signals result in percepts (perceptions), which the company attempted to balance in order to promote high consumer acceptance and continued use as opposed to rejection of the product.[89]

Pyrazines and learned behaviour

Pyrazine stimulation of olfactory receptors may enhance learned behaviour, either by acting alone or in combination with other sensory modality stimuli.[90 91] Human responses to chemosensory and olfactory effects that are associated with emotionally significant experiences can become constitutional through neuroplastic changes in the olfactory pathways to the limbic system as well as other areas of the brain associated with hedonic perception.[92 93] Such events can reinforce smoking through associative learning and become cues for increased hedonic valence of stimuli[94] and motivate increased desire or wanting, or even unrestrained consumption.

DISCUSSION

This is the first report to document the tobacco industry's incorporation of pyrazine compounds into cigarettes since the early 1970s which appear to contribute to the products' appeal and abuse potential. Effects of pyrazines in cigarettes as described in industry documents reflect a range of processes by which such non-nicotine constituents might increase tobacco product abuse potential.[84] Pyrazines may act in concert with nicotine either by chemosensory effects that reduce noxious sensations such as irritation in the upper ways and ease nicotine uptake and entry into the lung. They may also act by chemosensory effects that reinforce the learned behaviour of smoking, enhance elasticity and help optimise nicotine dosing to achieve a desired delivery to the brain and satisfy a smoker's need for the drug based on mood and circumstances.[95] Several pyrazine derivatives have also been found to potentiate 5-HT binding to receptors in the central nervous system, which results in enhanced dopamine release independently of nicotine.[96–98]

Chemosensory effects such as perceived smoothing and coolness (tactile) are associated with decreased aversion to smoking from the harshness and irritation of initial exposure to nicotine among novice smokers.[99 100] Similar effects have been described for menthol.[84] These effects might be a factor in smokers switching to 'low-tar' brands as an alternative to quitting smoking, going beyond the cognitive perception of reduced disease risk, to the emotive, physical perception that the smoke is 'smoother' and thus less harmful. Further, an RJR 1986 brand report describes the company's targeting of males 18–24 years of age by increasing the smoothness and masking the harshness and irritation of tobacco smoke.[101] The observed effects of pyrazines on secondhand smoke (SHS) demonstrate that these compounds were also used to reduce the irritation from SHS among non-smokers.[102] If non-smokers exposed to SHS perceive less risk due to lower irritation, without an actual reduction in their toxic constituents and effects, pyrazines might be classifiable as 'potentially hazardous constituents' under Section 904 of the Family Smoking and Tobacco Prevention Act of 2009 (FSPTCA).

Although independent research has been conducted on the effects on tobacco use of distal cueing from visual exposure to tobacco advertising and from social stimuli, little attention outside of tobacco manufacturers has previously been given to the more proximal cues that directly stimulate receptors of the head and neck.[89] The sensory inputs of pyrazine flavour additives might also provide cues for reward-related learned behaviours and could play a critical role in the development, maintenance and relapse of tobacco dependence. They could increase the attractiveness of smoking, particularly among youth.[103] Substantial evidence exists to suggest that flavour ingredients are used in cigarette 'starter' products, which increase cigarette experimentation and may help establish smoking behaviours that could lead to a lifetime of addiction.[103]

The FSPTCA explicitly bans the use of additives in cigarettes that are 'characterising flavours', which as defined by FDA food regulations are those that have taste or gustatory (eg, sweet, salt, sour, bitter) effects and are used in labelling, such as 'chocolate' flavoured cigarettes. However, a 'characterising' gustatory flavour may have relatively little significance if the major effect of an additive is on the olfactory and tactile receptors. British-American Tobacco concluded from research conducted that the prime sensorial experiences of smoking are associated with chemosensory flavour (odours, aroma) and irritation (tactile) sensations, whereas the gustatory qualities were found to be relatively less important for product attractiveness and appeal.[104] Flavour ingredients such as cocoa, licorice or vanilla have remained present in major cigarettes brands since prior to the ban's implementation,[54] which raises questions about the efficacy of the ban on the use of flavour ingredients and their consequential effects. When defining 'characterising' flavours for combusted tobacco products, the FDA Center for Tobacco Products should consider the distinction between flavours whose effects are primarily gustatory and flavours with olfactory or tactile effects.

Experimental use of electronic nicotine delivery systems (ENDS) has been rapidly increasing among teens.[105–108] Not surprisingly, the liquid flavour fluid formulations of ENDS include pyrazine additives such as 2,3,5,6-tetramethyl-pyrazine (0.9–1.5%), 2,3,5-trimethylpyrazine (0.3–4.5%) and acetylpyrazine (0.4–1.6%),[109] which also appear on the aforementioned lists of cigarette additives. Taken together, pyrazines appear to increase product appeal and make it easier for non-smokers to initiate smoking, more difficult for current smokers to quit, much easier for former smokers to relapse into smoking, and may mask the risks of both active and passive smoking.

The present findings should be interpreted in the context of the unique challenges of tobacco document research and known limitations with respect to documents availability. Access to the most recent industry documents is limited; use of terminology, practices and methods varies between companies and over time; and industry documents pertaining to pyrazines since the 1990s are largely unknown. Research conducted by industry is for business and commercial purposes, has not been peer reviewed and cannot be considered to be conclusive, absent independent confirmation. Therefore, a larger body of evidence should be considered with respect to the implications of these findings for public health and policy.

Future studies could focus on understanding the pivotal roles of pyrazines, their derivatives and other 'flavour' additives that stimulate neural receptors in neurobiological pathways, and actions in areas of the brain that affect abuse liability. Research could be conducted to examine the physiological and pharmacological actions of pyrazines and provide insight into the transduction mechanisms, receptor structure and chemical structure-activity relationships. Electrophysiological responses to chemosensory stimulants using radioactive labelled pyrazines and functional MRI and EEG could highlight specific areas of the brain stimulated by pyrazines.

The tobacco industry has long been interested in maximising the attractiveness, appeal, ease-of-use and low health-risk perceptions of tobacco products in a highly competitive and unregulated market in order to increase sales and market share.[29 36–38] To that end, manufacturers have researched and designed cigarettes with constituents that act independently of as well as synergistically with nicotine and may enhance abuse potential. The findings that are provided by these and other reports may help enable regulators such as the FDA, Health Canada, European Union and the WHO to develop standards to reverse these actions and reduce the addictiveness of tobacco products.

What this paper adds

- Nicotine is known as the drug that is responsible for the addicted behaviour of tobacco users, but it has been argued that non-nicotine factors are also essential to account for the intense addictive properties of tobacco smoking and high relapse rates among smokers after quitting.
- This study reveals how some tobacco manufacturers innovated with the use of pyrazines as additives. Pyrazines have been reported to have chemosensory and pharmacological properties and appear to be widely used now in cigarette brands.
- Pyrazines may help to optimise nicotine delivery and dosing, and promote addiction through cueing, learned behaviour and/or direct effects.

Disclaimer This research was conducted by the authors while at the Harvard School of Public Health. Dr Connolly is now Professor of Research at Northeastern University.

Contributors GNC had primary responsibility for the conception of the research. HRA, ITA and GNC contributed to the design of the research. HRA and ITA conducted the research and prepared drafts of the manuscript. GNC contributed to the editing of the manuscript drafts, and HRA prepared the final manuscript.

Funding This research was supported by the National Cancer Institute grant numbers 3R01 CA125224-03s1rev++ and 2R01 CA087477-09A2.

Competing interests ITA initiated his work on the reported research while affiliated with the Center for Global Tobacco Control at Harvard School of Public Health. He is currently affiliated with the Centers for Disease Control and Prevention's Office on Smoking and Health.

Provenance and peer review Not commissioned; externally peer reviewed.

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