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Tobacco particulate matter self-administration in rats: differential effects of tobacco type

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ABSTRACT

Nicotine self-administration in rats is the most widely used animal model of tobacco dependence. There is increasing evidence, however, that non-nicotinic constituents in smoke contribute to addiction and that different tobacco products contain varying levels of these constituents. The present study firstly sought to compare self-administration of pure nicotine to tobacco particulate matter (TPM) to determine if there were differences in reward-efficacy attributable to the non-nicotine constituents. Secondly, cigarette and roll-your-own (RYO) TPM groups were included and compared to determine whether different formulations of non-nicotinic constituents could impact reward. Briefly, male Sprague Dawley rats were implanted with indwelling jugular catheters for self-administration ($n = 76$). The reinforcing efficacy of infusions of nicotine (0.0 or 30.0 μg/kg/infusion) versus cigarette/RYO TPM (with matched nicotine content) was determined using spontaneous acquisition of self-administration on a fixed ratio schedule. The progressive ratio schedule was then employed to determine the motivation to receive each drug and within-subject dose–response curves were also produced (7.5, 15.0, 30.0 and 60.0 μg/kg/infusion nicotine). The main finding was that the RYO TPM was more reinforcing and produced a different profile of reward-related behaviour compared with both the nicotine and the cigarette TPM groups. The conclusions were that non-nicotinic components have a role in tobacco dependence and that some tobacco products could have higher abuse liability, irrespective of nicotine levels.

Keywords Cigarette, nicotine, roll-your-own, self-administration, tobacco dependence.

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INTRODUCTION

Smoking tobacco is the world's leading single preventable cause of mortality (Eriksen, Mackay & Ross 2012). Particularly dangerous is the addictive nature of smoking—making tobacco the most widely used substance of abuse. Smoking's addictive properties have been largely attributed to nicotine, a constituent present within the leaves and smoke (Benowitz 2009). Upon smoke inhalation, nicotine rapidly crosses the blood–brain barrier to bind acetylcholinergic receptors (nAChRs), located throughout the brain, which have the capacity to produce widespread alterations in neurotransmission (Clarke & Pert 1985; Brennan *et al.* 2010b). Of the many neurochemical effects, nicotine activates dopamine neurons in the ventral tegmental area (Pidoplichko *et al.* 1997) and increases dopamine overflow in the nucleus accumbens shell (Pontieri *et al.* 1996). These effects are strongly associated with the reinforcing effects of psychostimulant drugs (Di Chiara 2000) so, consequently, laboratory animals self-administer nicotine (Corrigall & Coen 1989).

Nicotine self-administration in rats is the most widely used animal model of human tobacco dependence (Le Foll & Goldberg 2009). Rats intravenously self-administer nicotine on a variety of schedules, but it has an unusual profile compared with other self-administered drugs. For instance, nicotine self-administration yields a relatively 'flat' dose–response curve, where responding is insensitive to changes in unit dose (Corrigall & Coen 1989; Donny *et al.* 1998; Manzardo, Stein & Belluzzi 2002; Harris, Pentel & LeSage 2009). This indicates that pharmacological mechanisms might not be the primary driver for nicotine self-administration. Further evidence to support this idea is that nicotine priming injections were relatively ineffective at reinstating drug-seeking in rats that had self-administered nicotine and then undergone extinction (LeSage *et al.* 2004; Feltenstein, Ghee & See 2012). This contrasts to other psychostimulant drugs, where a priming injection

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typically produces pronounced reinstatement (Schenk & Partridge 1999).

Nicotine is also weakly reinforcing when directly compared with prototypical drugs of abuse, such as cocaine. For example, rats selected cocaine over nicotine when provided a choice (Manzardo *et al.* 2002) and cocaine produced higher levels of responding on a progressive ratio (PR) schedule (Risner & Goldberg 1983). These experimental observations are difficult to reconcile with the highly addictive nature of tobacco smoking in humans. Possibly the pharmacological component of tobacco dependence is relatively minor and other factors such as social/environmental contributors are of equal or greater importance. Alternatively, nicotine alone might not accurately represent the pharmacology of smoke.

Tobacco smoke contains over 4000 chemical constituents (Stedman 1968), so perhaps using whole smoke extracts rather than nicotine alone would better model smoke pharmacology. An aqueous tobacco smoke extract or tobacco particulate matter (TPM) has recently been developed to these ends (Ambrose *et al.* 2007; Touiki *et al.* 2007; Harris *et al.* 2012; Lewis *et al.* 2012). When compared with matched doses of pure nicotine, TPM produced stronger inhibition of dorsal raphe serotonergic neurons (Touiki *et al.* 2007), differential nAChR expression in cell cultures (Ambrose *et al.* 2007) and monoamine oxidase (MAO) enzyme inhibition (Lewis *et al.* 2012). These findings indicate that TPM could produce a different self-administration profile to that of pure nicotine, which might better reflect the powerful nature of tobacco dependence.

Different types of tobacco display varying pharmacological properties and chemical composition (Ding *et al.* 2008; Lewis *et al.* 2012). For example, we have recently reported that roll-your-own (RYO) TPM exhibited significantly greater MAO inhibitory activity compared with cigarette TPM (Lewis *et al.* 2012). Analysis of the chemical composition revealed that RYO TPM contained double the concentration of harman and norharman when compared with cigarette TPM. Harman and norharman are psychoactive compounds that exert a range of neurological effects potentially affecting reward processes (Baum, Hill & Rommelspacher 1995, 1996; Touiki *et al.* 2005). Greater concentrations of these compounds in the RYO TPM might explain its higher MAO inhibitory activity, as these constituents are also known MAO inhibitors (Herraiz & Chaparro 2006). Harman and norharman produce a range of psychoactive effects and, because MAO inhibition could contribute to tobacco dependence (Guillem *et al.* 2006; Lewis, Miller & Lea 2007), RYO and cigarette tobacco might have differential abuse potential that is unrelated to nicotine content.

Comparisons between abuse liability of different tobacco products are highly relevant because the use of RYO tobacco is prevalent across many countries, including the United Kingdom, United States, Canada, Australia and New Zealand (Young *et al.* 2006, 2012). Between 2002 and 2008, incidence of predominant RYO use increased significantly in the United Kingdom and United States as a proportion of all cigarette use (Young *et al.* 2012). In many markets, RYO is the less expensive option and is subject to lower taxes—this is the main reason for its selection (Nosa *et al.* 2011; Young *et al.* 2012). RYO tobacco and cigarette paper manufacturers are also targeting a younger, trendy clientele, where RYO is touted as more 'natural/organic' and containing less 'chemicals' than factory-made cigarettes (Young *et al.* 2006).

Thus, the present study sought to compare the effects of nicotine and TPM in self-administration to examine for differences in reinforcing-efficacy, attributable to the non-nicotine components. Further, both a cigarette and a RYO TPM group were included to determine whether different formulations of non-nicotinic constituents could impact reinforcement.

MATERIALS AND METHODS

Subjects

Male Sprague Dawley rats were bred in the vivarium at Victoria University of Wellington ($n = 76$). The rats were maintained in a humidity- (77%) and temperature- (21°C) controlled animal housing facility on a 12-hour light/dark cycle (light: 7:00–19:00). They were housed in groups of four from weaning (21 days) until reaching weights suitable for surgery at approximately 300 g. The rats had unlimited access to food and water in the homecage prior to surgery and for duration of the 1-week recovery period. Upon commencement of self-administration testing, rats were fed 20 g of pellets (Diet 86, Sharpes Stockfeeds, Carterton, New Zealand) per day following each 2-hour session. This mild food restriction regimen maintains a gradual weight gain over time and has been utilized by many laboratories to facilitate nicotine self-administration (Corrigall & Coen 1989; Donny *et al.* 1998; LeSage *et al.* 2004; Guillem *et al.* 2005, 2006).

Apparatus

Testing was conducted in operant chambers (ENV 001, Med Associates, St. Albans, VT, USA) enclosed in sound-attenuating closets (ambient temperature of the testing room was 21°C). Each chamber contained two levers mounted 83 mm apart on one side of the operant chamber and 72 mm above the metal chamber floor grid. Depression of the right or (active) lever resulted in illumination of a stimulus light located above the lever and intravenous delivery of 0.25 ml of drug solution.

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Depression of the left (inactive) lever was recorded but produced no programmed consequence.

The self-administration parameters closely modelled those of Sorge & Clarke (2009), where there was a 30-second drug infusion time to more closely model the infusion of nicotine achieved when smokers inhale. There was a 'timeout' period of 120 seconds following each infusion earned, during which depression of the active lever did not deliver another infusion. Responses during timeout were recorded, but were not included in the total numbers of responses shown. Drug delivery and data acquisition was controlled by a Med-PC software package, and drug infusions were made via mechanical pumps (Razel, Model A with 1 rpm motor equipped with 20.0-ml syringes; Georgia, VT, USA).

Surgical procedures

Rats were implanted with intrajugular catheters under deep anaesthesia produced by an intraperitoneal injection of ketamine (90 mg/kg; Phoenix Pharm Distributors LTD, Auckland, New Zealand) and xylazine (9 mg/kg; Phoenix Pharm Distributors LTD). A small incision was made and the external jugular vein was isolated. The vein was tied off, a small cut was made and a silastic catheter was inserted and passed subcutaneously to an exposed portion of the skull where it was secured using dental acrylic adhered to four small jeweller's screws. The incision was closed with super glue and a topical antibiotic applied (Terramycin powder; Zoetis New Zealand LTD, Auckland, New Zealand). Immediately after surgery, each rat was administered 10 ml of sodium lactate solution via subcutaneous injection to prevent dehydration and aid recovery (Hartmann's solution, Baxter Healthcare, Old Toongabbie, Australia).

On each of two days following surgery, the analgesic and anti-inflammatory Carprofen® (5 mg /kg SC, Norbrook New Zealand LTD, Auckland, New Zealand) was administered. During the 7 days comprising the recovery period, catheters were flushed with 0.2 ml of a sterile 0.9% heparinized saline solution (30 UI/ml) containing penicillin G potassium (100 000 UI/ml). Catheter patency was tested at the start of the testing period and every week following the onset of the experiment (on Sundays) until the completion. Flushing the catheters with 0.15 ml of pentobarbitol (50.0 mg/kg, i.v., PROVET New Zealand, Auckland, New Zealand) and subsequent loss of the righting reflex within 5 seconds was indicative of patency. This regular test was critical because nicotine self-administration is notoriously resilient to extinction; thus, there is no immediate behavioural indication of a loss of patency. In the event of catheter patency failure, rats underwent repair surgery and were returned to the study following a 3-day post-surgery recovery period.

Self-administration procedures

The animals were placed in the operant chambers daily for 2-hour self-administration sessions (Monday–Friday). At the start of each session, the steel-tipped catheter on the head of the rat was attached to the tubing in the operant chamber that delivered drug. On the first day, the rats received three experimenter-administered primes and then two primes on the second day [modified methods based on Sorge & Clarke (2009)]. Thereafter, one prime was administered by the experimenter at the start of each session to fill the catheter with drug.

Rats were assigned to one of the following treatment groups: (1) vehicle control ($n = 16$); (2) nicotine (30 μg/kg/infusion; $n = 23$); (3) cigarette TPM (nicotine content 30 μg/kg/infusion; $n = 21$) or (4) RYO TPM (nicotine content 30 μg/kg/infusion; $n = 16$). The 30 μg/kg/infusion dose was selected because several previous reports indicate that it produces optimal levels of responding (Corrigall & Coen 1989; Donny *et al.* 1998), thus was intended to facilitate the acquisition process.

Self-administration was established on a fixed ratio (FR) schedule of reinforcement (days 1–10, FR1; days 11–15, FR2; days 16–25, FR5) (Guillem *et al.* 2006; Clemens *et al.* 2009). Self-administration was considered acquired if responding on the active lever was significantly greater than on the inactive and was greater than or equal to 20 responses on FR5 for at least 3 consecutive days. Rats that did not meet these criteria by the end of the FR5 period were excluded from further testing.

Dose–response testing was the next phase (on FR5), where all rats were allowed to self-administer each of four doses for 5 days (20 days in total) of nicotine, cigarette or RYO TPM. The average number of responses exhibited at each dose was computed for each rat. Rats completed the doses in a randomly assigned order. The full range of nicotine doses were 7.5, 15, 30 and 60 μg/kg/infusion. The cigarette/RYO TPM doses were matched for nicotine content.

A subset of rats completed PR schedule tests during the dose–response testing at each of the four doses. After the aforementioned 5 days of responding on a new dose, the rat would complete thee PR trials at that dose before changing to the next dose. The PR trials were conducted over 1 week, where each PR trial day was interspersed with a return to FR5 for 1 day. Each rat had to complete three PR trials to establish an average breakpoint. The PR schedule was based on the following equation: $5e^{(0.2 \times \text{Infusion\#})} - 5$ (rounded to the nearest integer), resulting in the following successive response requirements: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, etc. (Richardson & Roberts 1996). The PR sessions continued until 30 minutes had elapsed without a drug infusion.

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Drugs

(-)-Nicotine hydrogen tartrate salt (Sigma-Aldrich, Dorset, UK) was dissolved in sterile 0.9% saline to produce the starting training dose of 30 μg/kg/infusion (freebase), and adjusted to pH 7.2–7.4 with NaOH. The cigarette (Holiday brand) and RYO (Drum brand) TPM was prepared by Labstat International (Ontario, Canada) and solubilized in ethanol as previously described (Ambrose *et al.* 2007). TPM in ethanol was dried down as necessary under a stream of dry nitrogen, to 8 mM nicotine in ethanol. Then, 100 volumes of sterile isotonic saline was added and the resulting cloudy solution was shaken at 37°C overnight. Not all of the TPM components dissolved. The tarry residue was discarded and the nicotine, harman and norharman contents in the TPM solutions were quantified using mass spectrometric methods (Institute of Environmental Science & Research Ltd, Porirua, New Zealand).

These constituents were selected as being likely to affect reward-related behaviour and were measured to examine the reproducibility of the TPM solutions. This procedure produced equivalent levels of these constituents between batches. Cigarette TPM (nicotine 0.1 mg/ml, harman 0.13 μg/ml and norharman 0.26 μg/ml) and RYO TPM (nicotine 0.1 mg/ml, harman 0.27 μg/ml and norharman 0.54 μg/ml) were diluted with 1% ethanol saline to produce the starting-training dose of 30 μg/kg/infusion nicotine, where the pH was adjusted to 7.2–7.4 with NaOH when necessary. Note that because the TPM solutions contained approximately 1% ethanol, this proportion of ethanol was added to both the nicotine and the saline vehicle solutions.

Statistical analyses

The FR, dose response and PR breakpoints were analysed using repeated measures analyses of variance (ANOVAs) for a within-subject design. Specifically, sections of the FR experiment (FR1, FR2 and FR5) were considered separately, where within-subject factors were Day × Lever (active versus inactive) and the between-subjects factor was Treatment group. Dose was the within-subjects, and Treatment group the between-subjects factor for the FR5 dose response and average PR breakpoint data sets. *Post hoc* Bonferroni comparisons between Treatment groups were conducted when there were main ANOVA effects. Significance levels were set at 0.05.

RESULTS

FR schedule

During the first 10 days on FR1, responding changed significantly over time as there was a main effect of Day [$F(5,1296) = 28.145$, $P < 0.001$] (Fig. 1). A main effect

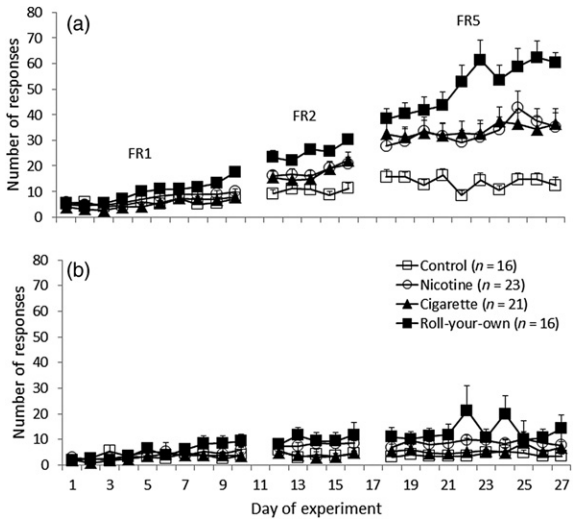

Figure 1 Self-administration on a fixed ratio (FR) schedule. Data points represent the average number of total responses produced by the nicotine, cigarette tobacco particulate matter (TPM) and roll-your-own TPM groups (FR1, days 1–10, FR2 days 12–16, FR5 days 18–27) on the active (a) or inactive (b) levers during daily 2-hour sessions (+SEM)

of Lever [$F(1,144) = 31.485$, $P < 0.001$] indicated an overall preference for the active lever, and this developed over time, as there was an interaction between Lever and Day [$F(5,1296) = 3.037$, $P < 0.01$]. There was no interaction between Lever and Treatment [$F(3,144) = 0.794$, not significant (NS)], showing a general preference for the active lever among all treatment groups. There was a main effect of Treatment condition on responding [$F(7,144) = 6.568$, $P < 0.001$], and an interaction between Treatment and Day [$F(16,1296) = 4.232$, $P < 0.001$]. *Post hoc* analyses revealed that these main effects were attributable to the RYO TPM group responding significantly more than controls ($P < 0.01$) and the cigarette TPM group ($P < 0.001$).

When the schedule was changed to FR2, the results were similar to those for FR1. There were main effects of Day [$F(3,572) = 8.860$, $P < 0.001$], Lever [$F(1,143) = 78.732$, $P < 0.001$], Treatment [$F(3,143) = 11.167$, $P < 0.001$] and a significant interaction between Day and Lever [$F(3,572) = 4.204$, $P < 0.01$]. *Post hoc* tests showed that the RYO TPM ($P < 0.001$) and nicotine ($P < 0.01$) groups were responding more than controls, and the RYO TPM group was responding at higher levels than the cigarette TPM ($P < 0.01$) and nicotine ($P < 0.05$) groups.

During the FR5 phase, there were main effects of Day [$F(6,1296) = 4.923$, $P < 0.001$], Lever [$F(1,144) = 127.268$, $P < 0.001$] and Treatment [$F(3,144) = 16.075$, $P < 0.001$] and an interaction between Day and Lever [$F(6,1296) = 3.771$, $P < 0.01$]. Furthermore, there were interactions between Day and Treatment [$F(17,1296) = 2.312$, $P < 0.01$] as responding was

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changing differentially over days as a function of treatment group. The number of active lever responses exhibited by the control group did not escalate as for the other groups; thus, there was a significant interaction between Treatment and Lever [$F(3,144) = 6.087$, $P < 0.01$]. The three-way interaction between Treatment, Lever and Day was significant at FR5, showing that all of these factors were changing over time and dependent on treatment condition [$F(17,1296) = 1.915$, $P < 0.05$]. *Post hocs* revealed that the nicotine, cigarette and RYO TPM were all responding significantly more than controls ($P < 0.01$). Further, the RYO TPM group exhibited responding that was also greater than the cigarette TPM and nicotine ($P < 0.01$) groups.

After the FR1 period (day 10), there were $n = 8$ nicotine and $n = 9$ cigarette TPM rats that did not meet acquisition criteria, whereas all the rats within the RYO TPM group had acquired self-administration. After the final day on FR5, there were only $n = 2$ rats in the nicotine and $n = 3$ in the cigarette TPM group that did not meet acquisition criteria. These observations show that generally, the present parameters yielded very high rates of self-administration for all groups. Although the RYO group was slightly smaller than the others, a more rapid acquisition was evident, where all rats had acquired self-administration by the end of the FR1 stage.

Dose–response curves

There were main effects of Dose [$F(3,96) = 5.441$, $P < 0.01$], Treatment [$F(2,32) = 7.927$, $P < 0.01$] and an interaction between these factors [$F(6,96) = 3.284$, $P < 0.01$] (Fig. 2a) for responding on the active lever. *Post hoc* analyses revealed that the RYO TPM group exhibited greater responding than the nicotine ($P < 0.01$) and cigarette TPM ($P < 0.05$) groups. Within-subject contrasts also revealed that RYO TPM responding at the 15 and 30 μg/kg/infusion doses were significantly greater than the other groups. On the inactive lever (Fig. 2b), there were no effects of Dose [$F(2,96) = 0.691$, NS], Treatment [$F(2,32) = 0.183$, NS] and no interaction between these factors [$F(5,96) = 0.674$, NS] (Fig. 3b).

There were main effects of Dose [$F(2,49) = 115.183$, $P < 0.001$], Treatment [$F(2,32) = 5.539$, $P < 0.01$], but no interaction between these factors [$F(3,49) = 2.384$, NS] for nicotine intake as a function of dose (Fig. 3c). *Post hoc* analyses revealed that RYO TPM group had a greater nicotine intake than the nicotine group ($P < 0.01$). Within-subject contrasts revealed that the RYO group had significantly greater nicotine intake at the 15 and 30 μg/kg/infusion doses.

PR schedule

The average breakpoint scores on the PR schedule were unaffected by dose changes, as there was no main effect of Dose [$F(2,45) = 2.379$, NS] and no interaction between Dose and Treatment [$F(4,45) = 1.882$, NS] (Fig. 3, top panel). There was an overall main effect of Treatment group on breakpoint scores [$F(2,15) = 5.336$, $P < 0.05$], where *post hoc* tests revealed that the RYO TPM group responded significantly more than the nicotine group on this schedule ($P < 0.05$) (Fig. 3, bottom panel).

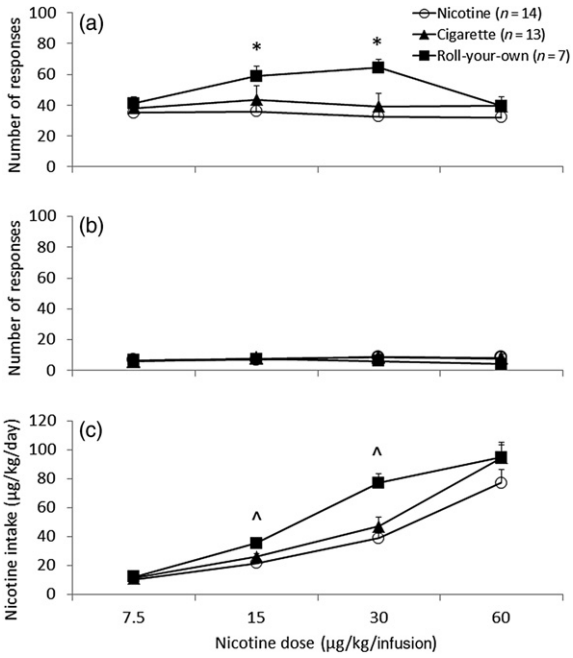

Figure 2 Dose–response curves on FR5. Data points represent the average number of active (a) and inactive (b) lever responses and nicotine intake (μg/kg/day) (c) produced at each dose of nicotine, cigarette and roll-your-own tobacco particulate matter (TPM) on FR5 (+SEM). The asterisk (*) indicates a significant difference between roll-your-own TPM and nicotine/cigarette TPM groups. The accent (^) indicates a significant difference between roll-your-own TPM and the nicotine group ($P < 0.05$)

DISCUSSION

Overview

The main finding was that the RYO TPM was more reinforcing and produced a different profile of reward-related behaviour compared with the nicotine and cigarette TPM groups.

Present results

During the first 10 days on the FR1 schedule, there was a general preference for the active lever among all treatment groups, including the controls (Fig. 1). As the vehicle solutions all contained 1% ethanol, the obvious possibility was that the ethanol had reinforcing effects. However, as we have previously observed that control rats with a saline vehicle showed this identical tendency

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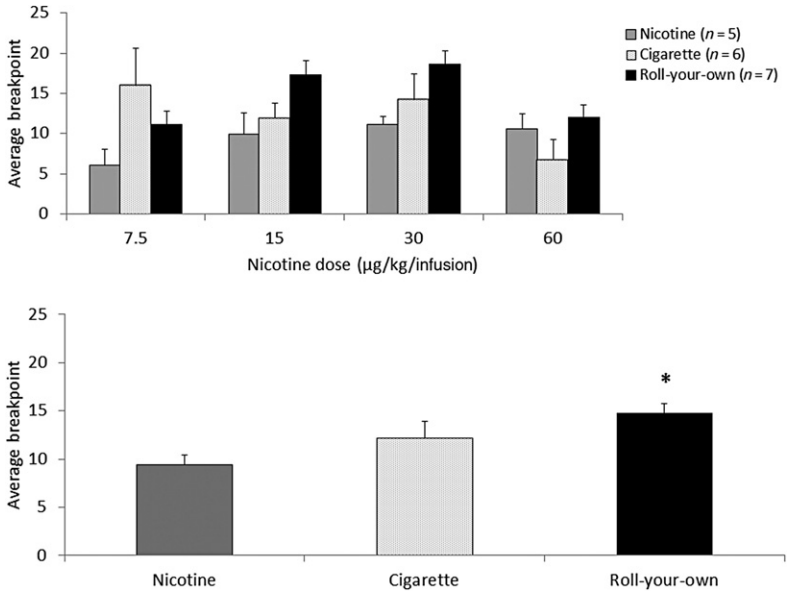

Figure 3 Self-administration on a progressive ratio (PR) schedule. Columns (top panel) represent the average breakpoint at each dose as a function of treatment group (+SEM). Columns (bottom panel) represent the average breakpoint collapsed across doses for each treatment group (+SEM). The asterisk (*) indicates a significant difference from the nicotine group ($P < 0.05$)

(unpublished data), it does not appear that the ethanol was responsible for the active lever preference. Rather, the most likely explanation is that the rats were motivated to respond for the light stimulus. The light stimulus is illuminated at the onset, and for the entire duration of each infusion, and it has been previously reported that a light stimulus alone is mildly reinforcing to rats (Sorge, Pierre & Clarke 2009).

The behavioural profile of nicotine and cigarette TPM self-administration was similar to the saline control during the FR1 phase. Only when the response requirement was increased to FR2/5 were these groups responding significantly more than controls. In contrast, the RYO TPM group were responding at significantly higher levels than controls during FR1 and throughout the FR2 and FR5 periods. Interestingly, rats rapidly acquire self-administration on FR1 of drugs with relatively high abuse potential, such as methamphetamine (Brennan *et al.* 2010a) or cocaine (Mandt *et al.* 2012). The present data indicate that the RYO TPM had acute reinforcing effects and exhibited a profile that was more comparable to reinforcing psychostimulant drugs.

When responding was assessed following several dose changes, the RYO TPM dose–response curve resembled the typical inverted U-shape, where highest responding occurred at the 15 and 30 μg/kg/infusion doses (Fig. 2). In contrast, the nicotine and cigarette TPM dose–response curves were flat, as has been previously described for nicotine. These results indicate that the RYO TPM group were adjusting intake according to dose changes, as might be expected when animals self-administer for the primary pharmacological effects of the drug.

These dose–response results correspond with the FR data (on the 30 μg/kg/infusion dose), where the 15 and 30 μg/kg/infusion doses yielded significantly higher rates of responding. Thus, the non-nicotinic components in the RYO TPM responsible for the increased responding produce optimal effects at the 15 and 30 μg/kg/infusion doses, whereas at the 7.5 and 60 μg/kg/infusion doses, the RYO TPM group was comparable to the other groups. This shows a relatively narrow window for the effectiveness of the non-nicotine components in influencing behaviour and demonstrates that the dose range selected for this study was optimal.

Increased responding by the RYO TPM group on the FR and dose–response tests were difficult to interpret alone. However, when combined with the PR results, it was evident that the rats were more motivated to acquire RYO TPM infusions (Fig. 3). The PR breakpoints were highest for the 15 and 30 μg/kg/infusion doses, which corresponds to the FR5 dose–response curve. Of interest, breakpoints for the cigarette TPM group were no different to the RYO TPM, or the nicotine groups—but it represented a 'halfway point' between the two. It is possible that whatever chemical constituents are responsible for the increased reward efficacy in the RYO group were also present, but perhaps at lower levels, in the cigarette TPM. In this way, the levels of the constituents responsible must exceed a certain threshold in order for the rewarding effects to become apparent.

One alternate explanation for the enhanced RYO TPM effect is that this TPM type had a different pharmacokinetic profile to nicotine/cigarette TPM. For example, faster clearance rates might explain increased levels of responding relative to the other groups if self-administering rats adjust responding to maintain blood levels of drugs (Lau & Sun 2002). This possibility would be more feasible if there had been differences between TPM (that contain a myriad of other chemicals) versus

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nicotine. However, there were no differences between the nicotine and cigarette TPM, which indicates that perhaps specific non-nicotinic components in the RYO were acting centrally rather than affecting metabolic/clearance processes.

Explanations and implications

Direct comparison between the cigarette TPM and nicotine groups revealed no significant differences across the present behavioural tests. This confirms the major role that nicotine has in tobacco dependence and shows that the particular formulation of non-nicotinic constituents in the present cigarette TPM solution did not significantly affect the reinforcing properties. In contrast, the RYO TPM had a very different profile. This indicates that the non-nicotinic agents in the RYO mixture significantly modified the reinforcing properties, rendering RYO TPM more like a prototypical drug of abuse. The most notable deviations to the usual nicotine-like profile were that responding was significantly higher than controls during FR1 and adjusted responding according to dose changes.

What non-nicotinic constituents could account for these differential effects between cigarette and RYO TPM? This study has provided evidence that cigarette and RYO tobacco have different reinforcing properties, thus substantially narrowing the search for these components. Firstly, harman and norharman were present at double the quantities in this RYO TPM compared with cigarette TPM, and the TPM solutions in the present study contained levels comparable to smoke levels (Pfau & Skog 2004). The neurological effects of these compounds include potentiation of dialysate dopamine levels in the nucleus accumbens in a dose-dependent manner (Baum *et al.* 1995, 1996). As dopamine levels in the accumbens have been closely associated with self-administration behaviour (Di Chiara 2000), it is possible that higher levels of these constituents in the RYO TPM enhanced the reinforcing properties of the mixture at select doses.

Alternatively, TPM might produce fewer serotonergic effects in proportion to dopaminergic effects, compared with nicotine alone. Generally, drugs that primarily increase serotonin neurotransmission do not maintain self-administration in animals (Schenk 2011) and those that release a greater proportion of serotonin compared with dopamine tend to be less reinforcing (Loh & Roberts 1990). However, drugs such as cocaine that release serotonin and dopamine in comparable proportions (Ravna, Sylte & Dahl 2003) are still strongly self-administered by animals, suggesting that serotonin-produced inhibitory effects can be masked, provided dopaminergic activation is sufficient (Schenk 2011). As intravenous doses of TPM have inhibited serotonergic neurons significantly more than matched doses of nicotine (Touiki *et al.* 2007), the RYO TPM might produce a greater (and more reinforcing) ratio of dopamine : serotonin.

Supporting this idea, nicotine and harman each exert considerable serotonergic inhibitory activity (Touiki *et al.* 2005). The combination of these agents might partly explain why TPM has stronger inhibitory activity than nicotine alone. As the present RYO solution contained much higher levels of harman, it follows that the RYO TPM might have had stronger inhibitory effects on serotonergic systems, thereby influencing serotonin/dopamine ratios.

RYO TPM also exhibits significantly greater MAO inhibitory activity, relative to the amount of nicotine, than cigarette TPM (Lewis *et al.* 2012). As MAO inhibition affects monoamine neurotransmission, it has been postulated to have a potential role in the development of tobacco dependence and possibly in the long-lasting persistence of cravings (Lewis *et al.* 2007). This hypothesis is difficult to study in humans, but animal self-administration experiments have demonstrated that pre-treatment with a range of different MAO inhibitor drugs has increased overall nicotine intake (Guillem *et al.* 2005, 2006; Villegier *et al.* 2007; Cohen, Koob & George 2012). Thus, constituents such as harman/norharman that inhibit MAO could contribute to the immediate reinforcing effects via this mechanism. However, there are other still unidentified candidates that could largely account for this effect. Harman and norharman are reversible inhibitors (Herraiz & Chaparro 2006), but the constituents in question are more likely to be irreversible inhibitors to produce the widespread and long-lasting MAO inhibition reported in smokers (Fowler *et al.* 2003).

Several other possible non-nicotinic constituents have been combined with nicotine or tested alone, such as acetaldehyde (Belluzzi, Wang & Leslie 2005), nornicotine (Bardo *et al.* 1999) or combinations of minor alkaloids (Clemens *et al.* 2009). These compounds have either significantly enhanced nicotine self-administration (Belluzzi *et al.* 2005; Clemens *et al.* 2009) or are self-administered alone (Bardo *et al.* 1999). It is highly probable that RYO TPM could contain higher levels of all these compounds, as well as unidentified ones, and that the behavioural effects could be attributed to synergistic action rather than to single constituents.

Thus, the reductionist approach of studying select compounds in isolation cannot account for the myriad of chemical interactions that are possible within tobacco smoke or a TPM solution. Consequently, future investigations could manipulate select constituents in the TPM, but keeping them within the whole smoke extract, to determine the effects on reinforcing efficacy in self-administration.

The implications of the present results are that smoking Drum RYO tobacco might be more addictive

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than Holiday cigarettes. It would be pre-mature to extrapolate these findings to all RYO and cigarette types, but this result does add to a more general concern around RYO tobacco. RYO tobacco has a significantly higher ratio of carcinogenic tar to nicotine than manufactured cigarettes and RYO smokers also tend to inhale more deeply (Laugesen *et al.* 2009), which might explain why RYO smokers are at greater risk of developing cancer of the oesophagus and larynx (Tuyns & Esteve 1983).

Interestingly, a large-scale survey (9046 participants) revealed that exclusive RYO smokers made fewer quit attempts than cigarette smokers and were less likely to succeed when they did (Young *et al.* 2006). These observations are in-line with the present study indicating that RYO tobacco has greater pharmacological addictive potential. As such, the increasing popularity and prevalence of RYO smoking means that greater regulation on taxation and advertising of RYO tobacco should be considered to mitigate population tobacco dependence and associated health consequences.

In conclusion, the present study is the first to demonstrate that rats will contingently self-administer a tobacco smoke extract, and that non-nicotinic agents in tobacco (RYO TPM) can significantly affect the reinforcing properties and susceptibility to relapse. The general implications are that some tobacco products could have higher abuse potential than others, irrespective of nicotine levels. Identifying the non-nicotine constituents that enhance the addictive capacity will enable tobacco products to be more accurately classified according to abuse liability and potential for harm.

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Conflict of interest

The authors declare no conflict of interest.

Authors contribution

KAB and PT were responsible for the study concept and design. KAB, AC, FP, VR and UW contributed to the acquisition of animal data. PT produced the TPM and supervised the analyses of constituent content. KAB, FP and PT assisted with data analysis and interpretation of findings. KB drafted the manuscript. PT provided critical revision of the manuscript for important intellectual content. All authors critically reviewed content and approved final version for publication.

References

Ambrose V, Miller JH, Dickson SJ, Hampton S, Truman P, Lea RA, Fowles J (2007) Tobacco particulate matter is more potent than nicotine at upregulating nicotinic receptors on SH-SY5Y cells. Nicotine Tob Res 9:793–799.

Bardo MT, Green TA, Crooks PA, Dwoskin LP (1999) Nornicotine is self-administered intravenously by rats. Psychopharmacology (Berl) 146:290–296.

Baum SS, Hill R, Rommelspacher H (1995) Norharman-induced changes of extracellular concentrations of dopamine in the nucleus accumbens of rats. Life Sci 56:1715–1720.

Baum SS, Hill R, Rommelspacher H (1996) Harman-induced changes of extracellular concentrations of neurotransmitters in the nucleus accumbens of rats. Eur J Pharmacol 314:75–82.

Belluzzi JD, Wang R, Leslie FM (2005) Acetaldehyde enhances acquisition of nicotine self-administration in adolescent rats. Neuropsychopharmacology 30:705–712.

Benowitz NL (2009) Pharmacology of nicotine: addiction, smoking-induced disease, and therapeutics. Annu Rev Pharmacol Toxicol 49:57–71.

Brennan KA, Colussi-Mas J, Carati C, Lea RA, Fitzmaurice PS, Schenk S (2010a) Methamphetamine self-administration and the effect of contingency on monoamine and metabolite tissue levels in the rat. Brain Res 1317:137–146.

Brennan KA, Lea RA, Fitzmaurice PS, Truman P (2010b) Nicotinic receptors and stages of nicotine dependence. J Psychopharmacol 24:793–808.

Clarke PB, Pert A (1985) Autoradiographic evidence for nicotine receptors on nigrostriatal and mesolimbic dopaminergic neurons. Brain Res 348:355–358.

Clemens KJ, Caille S, Stinus L, Cador M (2009) The addition of five minor tobacco alkaloids increases nicotine-induced hyperactivity, sensitization and intravenous self-administration in rats. Int J Neuropsychopharmacol 12:1355–1366.

Cohen A, Koob GF, George O (2012) Robust escalation of nicotine intake with extended access to nicotine self-administration and intermittent periods of abstinence. Neuropsychopharmacology 37:2153–2160.

Corrigall WA, Coen KM (1989) Nicotine maintains robust self-administration in rats on a limited-access schedule. Psychopharmacology (Berl) 99:473–478.

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Di Chiara G (2000) Role of dopamine in the behavioural actions of nicotine related to addiction. Eur J Pharmacol 393:295–314.

Ding YS, Zhang L, Jain RB, Jain N, Wang RY, Ashley DL, Watson CH (2008) Levels of tobacco-specific nitrosamines and polycyclic aromatic hydrocarbons in mainstream smoke from different tobacco varieties. Cancer Epidemiol Biomarkers Prev 17:3366–3371.

Donny EC, Caggiula AR, Mielke MM, Jacobs KS, Rose C, Sved AF (1998) Acquisition of nicotine self-administration in rats: the effects of dose, feeding schedule, and drug contingency. Psychopharmacology (Berl) 136:83–90.

Eriksen M, Mackay J, Ross H (2012) *The Tobacco Atlas (Revised 4th Ed)*. Available at: http://www.tobaccoatlas.org/. Accessed 25 September 2013.

Feltenstein MW, Ghee SM, See RE (2012) Nicotine self-administration and reinstatement of nicotine-seeking in male and female rats. Drug Alcohol Depend 121:240–246.

Fowler JS, Logan J, Wang GJ, Volkow ND (2003) Monoamine oxidase and cigarette smoking. Neurotoxicology 24:75–82.

Guillem K, Vouillac C, Azar MR, Parsons LH, Koob GF, Cador M, Stinus L (2005) Monoamine oxidase inhibition dramatically increases the motivation to self-administer nicotine in rats. J Neurosci 25:8593–8600.

Guillem K, Vouillac C, Azar MR, Parsons LH, Koob GF, Cador M, Stinus L (2006) Monoamine oxidase A rather than monoamine oxidase B inhibition increases nicotine reinforcement in rats. Eur J Neurosci 24:3532–3540.

Harris AC, Pentel PR, LeSage MG (2009) Correlates of individual differences in compensatory nicotine self-administration in rats following a decrease in nicotine unit dose. Psychopharmacology (Berl) 205:599–611.

Harris AC, Stepanov I, Pentel PR, Lesage MG (2012) Delivery of nicotine in an extract of a smokeless tobacco product reduces its reinforcement-attenuating and discriminative stimulus effects in rats. Psychopharmacology (Berl) 220:565–576.

Herraiz T, Chaparro C (2006) Human monoamine oxidase enzyme inhibition by coffee and beta-carbolines norharman and harman isolated from coffee. Life Sci 78:795–802.

Lau CE, Sun L (2002) The pharmacokinetic determinants of the frequency and pattern of intravenous cocaine self-administration in rats by pharmacokinetic modeling. Drug Metab Dispos 30:254–261.

Laugesen M, Epton M, Frampton CM, Glover M, Lea RA (2009) Hand-rolled cigarette smoking patterns compared with factory-made cigarette smoking in New Zealand men. BMC Public Health 9:194.

Le Foll B, Goldberg SR (2009) Effects of nicotine in experimental animals and humans: an update on addictive properties. Handb Exp Pharmacol 192:335–367.

LeSage MG, Burroughs D, Dufek M, Keyler DE, Pentel PR (2004) Reinstatement of nicotine self-administration in rats by presentation of nicotine-paired stimuli, but not nicotine priming. Pharmacol Biochem Behav 79:507–513.

Lewis A, Miller JH, Lea RA (2007) Monoamine oxidase and tobacco dependence. Neurotoxicology 28:182–195.

Lewis AJ, Truman P, Hosking MR, Miller JH (2012) Monoamine oxidase inhibitory activity in tobacco smoke varies with tobacco type. Tob Control 21:39–43.

Loh EA, Roberts DC (1990) Break-points on a progressive ratio schedule reinforced by intravenous cocaine increase following depletion of forebrain serotonin. Psychopharmacology (Berl) 101:262–266.

Mandt BH, Johnston NL, Zahniser NR, Allen RM (2012) Acquisition of cocaine self-administration in male Sprague-Dawley rats: effects of cocaine dose but not initial locomotor response to cocaine. Psychopharmacology (Berl) 219:1089–1097.

Manzardo AM, Stein L, Belluzzi JD (2002) Rats prefer cocaine over nicotine in a two-lever self-administration choice test. Brain Res 924:10–19.

Nosa V, Glover M, Min S, Scragg R, Bullen C, McCool J, Kira A (2011) The use of the 'rollie' in New Zealand: preference for loose tobacco among an ethnically diverse low socioeconomic urban population. N Z Med J 124:25–33.

Pfau W, Skog K (2004) Exposure to beta-carbolines norharman and harman. J Chromatogr B Analyt Technol Biomed Life Sci 802:115–126.

Pidoplichko VI, DeBiasi M, Williams JT, Dani JA (1997) Nicotine activates and desensitizes midbrain dopamine neurons. Nature 390:401–404.

Pontieri FE, Tanda G, Orzi F, Di Chiara G (1996) Effects of nicotine on the nucleus accumbens and similarity to those of addictive drugs. Nature 382:255–257.

Ravna AW, Sylte I, Dahl SG (2003) Molecular mechanism of citalopram and cocaine interactions with neurotransmitter transporters. J Pharmacol Exp Ther 307:34–41.

Richardson NR, Roberts DC (1996) Progressive ratio schedules in drug self-administration studies in rats: a method to evaluate reinforcing efficacy. J Neurosci Methods 66:1–11.

Risner ME, Goldberg SR (1983) A comparison of nicotine and cocaine self-administration in the dog: fixed-ratio and progressive-ratio schedules of intravenous drug infusion. J Pharmacol Exp Ther 224:319–326.

Schenk S (2011) MDMA ('ecstasy') abuse as an example of dopamine neuroplasticity. Neurosci Biobehav Rev 35:1203–1218.

Schenk S, Partridge B (1999) Cocaine-seeking produced by experimenter-administered drug injections: dose-effect relationships in rats. Psychopharmacology (Berl) 147:285–290.

Sorge RE, Clarke PB (2009) Rats self-administer intravenous nicotine delivered in a novel smoking-relevant procedure: effects of dopamine antagonists. J Pharmacol Exp Ther 330:633–640.

Sorge RE, Pierre VJ, Clarke PB (2009) Facilitation of intravenous nicotine self-administration in rats by a motivationally neutral sensory stimulus. Psychopharmacology (Berl) 207:191–200.

Stedman RL (1968) The chemical composition of tobacco and tobacco smoke. Chem Rev 68:153–207.

Touiki K, Rat P, Molimard R, Chait A, de Beaurepaire R (2005) Harmane inhibits serotonergic dorsal raphe neurons in the rat. Psychopharmacology (Berl) 182:562–569.

Touiki K, Rat P, Molimard R, Chait A, de Beaurepaire R (2007) Effects of tobacco and cigarette smoke extracts on serotonergic raphe neurons in the rat. Neuroreport 18:925–929.

Tuyns AJ, Esteve J (1983) Pipe, commercial and hand-rolled cigarette smoking in oesophageal cancer. Int J Epidemiol 12:110–113.

Villegier AS, Lotfipour S, McQuown SC, Belluzzi JD, Leslie FM (2007) Tranylcypromine enhancement of nicotine self-administration. Neuropharmacology 52:1415–1425.

Young D, Borland R, Hammond D, Cummings KM, Devlin E, Yong HH, O'Connnor RJ (2006) Prevalence and attributes of roll-your-own smokers in the International Tobacco Control (ITC) Four Country Survey. Tob Control 15 (Suppl. 3):iii76–iii82.

Young D, Yong HH, Borland R, Shahab L, Hammond D, Cummings KM, Wilson N (2012) Trends in roll-your-own smoking: findings from the ITC Four-Country Survey (2002–2008). J Environ Public Health 2012:406283.

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