Contribution of Harman and Norharman to the reinforcing efficacy of aqueous tobacco smoke extract self-administered by rats

by

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A thesis

submitted to the Victoria University of Wellington

in fulfilment of the requirements for the degree of

Masters of Science

Victoria University of Wellington

(2012)

Abstract

Background: Animal models of drug abuse treat nicotine as the primary reinforcing agent that promotes tobacco addiction. However, rodents demonstrate poor self-administration of nicotine despite evidence of tobacco's high abuse potential in humans. This discrepancy has been attributed to other constituents of tobacco smoke that facilitate the development of nicotine dependence.

Objectives: This study aimed to determine whether rats would self-administer intravenous an aqueous tobacco smoke extract (TPM) to find evidence if it was more reinforcing than nicotine alone. The study also evaluated the role of tobacco smoke constituent's harman and norharman in any differences observed.

Methods: Firstly, male Sprague-Dawley rats (n=29) were assigned to treatment groups: nicotine (30.0μg/kg/infusion), TPM (containing 30.0μg/kg/infusion nicotine) or saline vehicle. Ability for each treatment to support intravenous self-administration was assessed using spontaneous acquisition of responding on gradually increasing fixed ratio schedules (FR1, FR2, FR5). Subsequent progressive ratio (PR) testing was employed to determine reinforcing efficacy of each treatment. Then a second group of rats (N = 56) were assigned to treatment groups: nicotine alone (30.0 or 75.0μg/kg/infusion) or nicotine combined with norharman (0, 0.4, 2.5 or 6.25μg/kg/infusion) and harman (0.0, 1.6 or 10.0μg/kg, IP), and tested using a similar protocol.

Results: Animals readily acquired self-administration responding for TPM and produced higher PR breakpoints (BP) than rats treated with nicotine alone or vehicle. Rats trained to respond for a larger dose of nicotine demonstrated significantly greater response rates than those receiving the lower dose of nicotine. Finally, the addition of harman and norharman to

nicotine significantly reduced BP at the lower unit dose of nicotine tested.

Conclusions: These findings support the hypothesis that TPM is more reinforcing than nicotine alone. However, the increased reinforcing efficacy of TPM cannot be attributed to the actions of harman and norharman. The potential role of serotonin inhibition in tobacco reward processes is discussed.

Introduction

Tobacco addiction is considered the prototypical substance abuse condition (US DHHS, 1988) with roughly one quarter of the world's adult population currently addicted to tobacco products (WHO, 2008). For western populations the prevalence of tobacco dependence far outstrips that of other substance disorders as defined by DSM criteria (Anthony *et al.*, 1994; Kandel *et al.*, 1997). In New Zealand alone approximately 20% of the population self-report regular use of tobacco products, with a higher rate of use amongst youth populations (WHO, 2009). Up to 75% of cigarette smokers express a desire to discontinue use, but only 2 - 5% of smokers are able to stop using tobacco without the aid of additional therapies (Okuyemi *et al.*, 2000). Perhaps even more startling is that following operations to repair damage done by chronic use of cigarettes such as lung surgery and laryngectomy, roughly 40% of people resume smoking (Stolerman & Jarvis, 1995).

Tobacco smoke contains thousands of chemicals, many of which are known to have actions in the central nervous system (CNS; Shiffman *et al.*, 1998). Nicotine is widely recognised as the chemical in tobacco products responsible for their addictive properties (US DHHS, 1988; Stolerman & Jarvis, 1995). For example, currently addicted smokers will reliably self-administer nicotine intravenously when cigarettes are not available (Harvey *et al.*, 2004) and rate intravenous nicotine delivery as a positive experience. Intravenously administered nicotine also reproduces many of tobacco's peripheral and euphoric effects (Henningfield *et al.*, 1985). Direct administration of nicotine will alleviate the withdrawal symptoms that result from prolonged tobacco abstinence which include increased anger, anxiety, impatience, restlessness, difficulty concentrating and increased hunger (Hughes *et al.*, 1991). When the actions of nicotine on the CNS are blocked pharmacologically, smokers will

increase their intake in an effort to compensate for reduced nicotine efficacy (Stolerman *et al.*, 1973). Finally, the use of nicotine replacement therapy (NRT) during withdrawal has been shown to double the rate of successful abstinence from tobacco use for up to 12 months (Hughes *et al.*, 1991).

Due to the limitations inherent in human research, various animal models of nicotine dependence have been utilised to study the addiction related pharmacological actions of nicotine. Self-administration protocols, which involve the intravenous infusion of a bolus of drug contingent upon an operant response, have been used to characterise addictive properties of most drugs abused by humans (Di Chiara & Imperato, 1988). Similarly, reliable intravenous self-administration of nicotine has been established for multiple animal species including non-human primates (Goldberg *et al.*, 1981), dogs (Risner & Goldberg, 1983), mice (Martellotta *et al.*, 1995; Picciotto *et al.*, 1998) and rats (Corrigall & Coen, 1989; Donny *et al.*, 1995). In rodents, the rate of responding maintained by nicotine is higher in rats than in mice (Le Foll & Goldberg, 2006, pg 371). Rats have been shown to be sensitive to the discriminative properties of nicotine (Stolerman *et al.*, 1984) and its ability to act as an unconditioned stimulus in conditioned place preference tests (Le Foll & Goldberg, 2005). Additionally, nicotine administration produces an effect on brain reward circuitry, as indicated by reduced intracranial self-stimulation (ICSS) thresholds following administration (Pradhan & Bowling, 1971).

Different schedules of reinforcement are used in self-administration protocols in an attempt to characterise different aspects of a substance's reinforcing effects. Progressive ratio (PR) schedules involve implementation of a response requirement which gradually increases after each infusion that is earned. The final ratio completed before responding ceases is termed the breakpoint (BP) and has been suggested to provide a better quantitative measure of a

drug's reinforcing efficacy than responding emitted under fixed-ratio (FR) schedules of reinforcement (Richardson & Roberts, 1996; Stafford *et al.*, 1998). To date, reliable self-administration of various substances abused by humans has been demonstrated under a PR schedule of reinforcement (Le Foll & Goldberg, 2006) including nicotine (Donny *et al.*, 1999).

Nicotine acts on the CNS via nicotinic acetylcholine receptors (nAChR; Brennan *et al.*, 2010). nAChRs are pentameric ligand-gated ion channels composed of a variety of subunits (α2 - α10, β2 - β4) in varying configurations (Wonnacott *et al.*, 2005). Nicotine's affinity for any given nAChR is related directly to the receptors subunit composition (Pidoplichko *et al.*, 1997). Several nicotine-produced behaviours are attenuated by nAChR antagonists and thus are reliant upon nAChR activation: nicotine induced locomotor activation (Benwell *et al.*, 1995), lowered ICSS thresholds (Pradhan & Bowling, 1971; Harrison *et al.*, 2002) and drug discrimination (Stolerman *et al.*, 1984). Additionally, the nAChR partial agonists sazetidine-A and varenicline effectively substitute for nicotine in the drug discrimination paradigm, and are self-administered by animals (Patterson *et al.*, 2010). Pre-treatment with the nAChR antagonist mecamylamine during self-administration sessions attenuates responding in a similar fashion to saline substitution of the nicotine reinforcer (Corrigall & Coen, 1991; Martellotta *et al.*, 1995; Shoaib *et al.*, 1997; Donny *et al.*, 1999). Further, mecamylamine is also known to precipitate the aversive withdrawal symptoms experienced by nicotine dependent animals (Watkins *et al.*, 2000). Thus it seems that nicotine's effects on addiction related brain mechanisms occur via its actions on nAChRs.

The mesolimbic dopamine (DA) system plays a vital role in the reinforcing and addictive properties of addictive drugs such as cocaine, heroin and amphetamine (Wise & Bozarth, 1987). Further, nAChRs have been localised to mesolimbic DA containing neurons

in the ventral tegmental area (VTA; Calabresi *et al.*, 1989) and their terminal fields in the nucleus accumbens (NAc; Pontieri *et al.*, 1996). Acute activation of nAChRs located on VTA cell bodies causes excitatory release of DA into the NAc (Corrigall, 1999) that was similar to the DA efflux caused by other drugs of abuse (Di Chiara & Imperato, 1988). By comparison, chronic activation of nAChRs leads to a desensitisation of nicotine's ability to stimulate DA efflux (Pidoplichko *et al.*, 1997), a process believed to result from a functional upregulation of nAChRs (Parker *et al.*, 2004). Changes in the ability of nicotine to elicit DA efflux in the NAc as a result of chronic exposure has been suggested to underlie the development of nicotine dependence (Di Chiara, 2000; Rahman *et al.*, 2004). In particular changes in the expression of the α4 (Tapper *et al.*, 2004), β2 (Picciotto *et al.*, 1998), α6 (Parker *et al.*, 2004) and α7 (Schilstrom *et al.*, 1998) nAChR subunits have been suggested as central to nicotine's long lasting effects on mesolimbic DA reward circuitry.

Similar to other drugs of abuse, the ability of nicotine to act as a reinforcer is directly related to its ability to stimulate DA release into the NAc (Di Chiara, 2000). Supporting this idea, nicotine reinforced self-administration was suppressed by pretreatment with the non-selective DA receptor antagonist haloperidol, as well as by selective D1- and D2-like receptor antagonists (Corrigall & Coen, 1991). Selective antagonism of the D1-like receptor has also blocked nicotine-produced decreases in ICSS thresholds (Harrison *et al.*, 2002). When dopaminergic neurons in the VTA were lesioned using 6-hydroxydopamine, self-administration of nicotine was attenuated and response patterns became similar to those exhibited during extinction sessions (Corrigall *et al.*, 1992). Finally, a variety of DA receptor agonists will effectively substitute for nicotine, including other drugs of abuse like cocaine and the amphetamines (Di Chiara, 2000).

Despite the common effects produced by nicotine and other drugs of abuse on

mesolimbic DA reward systems, the ability of nicotine to stimulate DA neuron activity is less pronounced (Sziraki *et al.*, 1999). Nicotine's reduced ability to stimulate DA has been indicated whereby when given a choice, rats show a consistent preference to self-administer cocaine over nicotine (Manzardo *et al.*, 2002). Furthermore, acquisition of nicotine self-administration is only achieved when using stricter conditions than is common for other substances. For example, prior training in operant procedures with a food reinforcer and food restricting the animals is used to facilitate acquisition of nicotine self-administration (Corrigall, 1992). Even once nicotine is reliably self-administered it produces less robust self-administration than cocaine (Spealman & Goldberg, 1982) and fails to show multiple phenomena common to other drugs of abuse. For example, nicotine self-administration demonstrates considerably less sensitivity to dose than cocaine (Lynch & Carroll, 1999). Instead nicotine produces flat dose response curves, which are insensitive to changes in the unit dose (Corrigall *et al.*, 1989; Corrigall, 1992).

The weaker reinforcing effects exhibited by nicotine in animal models of drug use have contributed to the claim that nicotine is not an addictive substance (Robinson & Pritchard, 1992; Dar & Frenk, 2004). Another commonly utilised argument against nicotine as being addictive involves the overall low efficacy of NRT treatments. Although NRT doubles the overall rate of successful smoking abstinence, this effect is short lasting, with at least 80% of people that used NRT resuming smoking in 6 months (Shiffman *et al.*, 1998). Studies have also shown that while NRT alleviates withdrawal symptoms experienced during tobacco abstinence, denicotinised cigarettes were substantially more effective (Rose *et al.*, 2000). Also, arguing against nicotine as an addictive substance, the rate of pure nicotine abuse or dependence reported amongst both smokers and non-smokers was miniscule (Hughes *et al.*, 1991). Even in cases where NRT was continued to be used beyond the recommended

cessation date, all prolonged users were effectively weaned off of NRT products (West *et al.*, 2000). These findings suggest that nicotine alone cannot explain or accurately model the pharmacological aspect of tobacco dependence.

There is growing evidence that there are non-nicotinic constituents in tobacco that contribute to reward. Various tobacco constituents have been tested alone, and in combination with nicotine to determine whether any unique interactions existed regarding reinforcement processes. Nornicotine, a metabolite of nicotine, which also exists in tobacco smoke, supported stable, although weaker, self-administration responding than that produced by nicotine (Bardo *et al.*, 1999). The tobacco constituent acetaldehyde enhanced nicotine reinforced self-administration behaviour in young rats only, without demonstrating any reinforcing value alone (Belluzzi *et al.*, 2005). Clemens *et al.* (2009) tested a cocktail of tobacco alkaloids together with nicotine (anabasine, nornicotine, anatabine, cotinine and myosmine) and found similar synergistic actions. When combined with nicotine, the cocktail of alkaloids increased the development of locomotor sensitisation. Further, motivation to respond for the tobacco alkaloid and nicotine cocktail was greater than that for nicotine alone when measured using a PR schedule of reinforcement. Finally, addition of the tobacco alkaloids produced an inverted U-shaped dose response curve for nicotine similar to those reported for other drugs of abuse.

Tobacco smoke has produced differential addiction related neuroadaptations when compared to nicotine alone. Firstly, there are non-nicotine constituents in tobacco smoke that can also bind nAChRs to produce effects on dopaminergic neurotransmission. For example, the tobacco constituents nornicotine, anabeseine, anabesine and N-methylanabasine all stimulated DA efflux and produced desensitisation of nAChRs in the striatum *in vitro* (Dwoskin *et al.*, 1995) while the tobacco constituent cotinine specifically activated and

upregulated nAChRs containing the α3 and α6 subunits (O'Leary *et al.*, 2008). Exposure to aqueous tobacco smoke extract suppressed the activity of serotonin (5-HT) neurons in the raphe nucleus to a greater extent than a matched dose of nicotine alone (Touiki *et al.*, 2007). Cell culture work revealed that aqueous tobacco extract produced a greater upregulation in nAChR when compared to matched doses of nicotine alone (Ambrose *et al.*, 2007). Finally, rats chronically exposed to tobacco smoke showed a reduction in response latencies in ICSS paradigms which were not detected following nicotine treatment (Small *et al.*, 2010). These studies are indicative that tobacco has psychostimulant properties that do not involve a nicotine component.

Of the candidate tobacco constituents likely to play a role in the additional effects of tobacco smoke compared to nicotine, monoamine oxidase enzyme (MAO) inhibitors have gathered particular interest. There are two currently identified forms of central MAO enzymes, A and B, which both function to break down monoamines such as DA and 5-HT. Tobacco smoke contains chemicals that block the actions of MAO, termed monoamine oxidase inhibitors (MAO-I). Positron emission tomography has revealed that MAO availability in the brains of current smokers was significantly less for both MAO-A (Fowler *et al.*, 1996a) and MAO-B (Fowler *et al.*, 1996b). Reduced functionality of MAO-B has also been linked to an upregulation of MAO-B proteins in smokers up to 15 years after quitting (Launay *et al.*, 2009). These effects cannot be attributed to the actions of nicotine as it does not inhibit MAO activity at physiologically relevant levels (Fowler *et al.*, 1998). Since MAO inhibition can alter dopaminergic and serotonergic neurotransmission (Lewis *et al.*, 2007), this smoke-produced neuroadaptation is likely to impact reward.

This idea is supported by experiments showing that rats are more willing to respond for nicotine on both FR and PR schedules of reinforcement when pre-treated with MAO-I

(Guillem *et al.*, 2005). Because MAO-I had no effect on food reinforced responding, and were less effective at enhancing nicotine self-administration in rats demonstrating low response to novelty (Guillem *et al.*, 2005), it was suggested that MAO-I might potentiate nicotine-produced sensitisation of DA circuitry. In support of this idea, nicotine produced behavioural sensitisation as measured by locomotor activation was sustained for substantially longer following MAO-I treatment (Villegier *et al.*, 2003).

The β -carbolines (harman and norharman), are MAO-I that exist in a variety of commonly consumed products, including coffee, cooked meat and tobacco smoke (Pfau & Skog, 2004; Herraiz, 2004). Of these, tobacco smoke provides a particularly rich source of these β-carbolines and recent evidence has suggested these constituents could be largely responsible for tobacco's MAO-I effects (Van Amsterdam *et al.*, 2006). Harman and norharman levels rise in blood plasma after smoking (Breyer-Pfaff *et al.*, 1996; Rommelspacher *et al.*, 2002; Spijkerman *et al.*, 2002) and readily cross the blood brain barrier (Fekkes & Bode, 1993; Rommelspacher *et al.*, 1994). Additionally, both harman and norharman accumulated in the blood platelets of smokers after cigarette consumption (Rommelspacher *et al.*, 2002) and have been shown to potently suppress MAO-A and MAO-B activity *in vivo* (Herraiz & Chaparro, 2005). Specifically, harman selectively inhibited MAO-A, while norharman inhibited both MAO forms but with lower affinity than harman.

Harman and norharman exert neuronal effects beyond, and in addition to, MAO inhibition. Both of these compounds are highly active in the CNS. They activate adrenergic neurons in the locus coeruleus (Ruiz-Durantez *et al.*, 2001) and mesolimbic DA neurons in the NAc (Ergene & Schoener, 1993). In conjunction with increased dopaminergic neuronal firing, systemic administration of harman (Baum *et al.*, 1996) and norharman (Baum *et al.*, 1995) altered DA dialysate levels in the rat NAc. Harman affects 5-HT systems, where systemic

injection produced long-lasting inhibition of dorsal raphe serotonergic neurons (Touiki *et al.*, 2005) and local administration increased 5-HT efflux in the hippocampus (Adell *et al.*, 1996). Because the psychoactive compounds harman and norharman are present in relatively large quantities in tobacco smoke, there is a need to determine whether they contribute to the development of tobacco dependence.

The first aim of the present study was to determine whether an aqueous tobacco smoke extract (tobacco particulate matter; TPM) can be intravenously self-administered by rats, and whether there is evidence that it is more rewarding than nicotine alone. This was accomplished by comparing self-administration of nicotine to a TPM solution with a matched nicotine concentration. Reinforcing efficacy of intravenous infusions of nicotine or TPM were determined using spontaneous acquisition of self-administration on a fixed ratio (FR) schedule. The progressive ratio (PR) schedule was then employed to determine the motivation to receive each drug. The second aim was to determine whether harman and norharman would alter the rewarding properties of self-administered nicotine. Thus, doses of harman and norharman were selected that were within the realms of what a smoker might be exposed to in a day and similar testing procedures were used as above to compare behaviour between nicotine alone and nicotine combined with harman and norharman treatment groups.

Method

Animals

Experimentally naive male albino Sprague-Dawley rats bred in the vivarium at Victoria University of Wellington were used for this experiment (n = 102). Animals underwent surgery once they reached weights between 300-330g and were subsequently housed individually in

26x20x42cm Plexiglas home cages lined with pine woodchip bedding. Home cages were kept at 21±1 ° Celsius with 77% humidity and maintained on a 12hr light/dark cycle (light: 7:00am - 7:00pm). Water was made available ad-libitum in the home cage while access to rat chow was limited to 20g of pellets (Diet 86; Sharpes, New Zealand) per day available following the completion of the self-administration session. This feeding schedule has been used successfully by previous researchers to encourage acquisition of nicotine self-administration without depriving animals of dietary requirements (Corrigall, 1992; Lynch & Carroll, 1999; Guillem *et al.*, 2005, 2006; Clemens *et al.*, 2009). The experiment was run with approval from the Victoria University of Wellington Animal Ethics Committee and all procedures were consistent with the New Zealand Animal Welfare Act 1999.

Apparatus

Self-administration sessions were conducted in 28x21x21cm sized operant chambers (Med Associates, ENV-001) enclosed by attenuating closets maintained at 21 ° Celsius and controlled using a Med-PC software package (Med Associates, Med-PC IV). Each chamber also contained a syringe pump (Razel, Model A containing a 20.0ml syringe equipped with a 230v 1rpm motor) responsible for intravenous delivery of drug treatment via silastic tubing. The tubing was protected by a spring leash (Plastics One, USA), which was anchored to the animal's skullcap screw. The tubing was suspended above the animal and attached to a freely moving swivel (Harvard Apparatus, USA), which allowed for free movement within the chamber. Two levers were mounted 83mm apart on a single side of the operant chamber and 72mm above the chamber floor, which included metal rods elevated above a layer of wood shavings. Depression of the right (active) lever resulted in delivery of 0.25ml of treatment solution accompanied by illumination of a stimulus light located directly above the active

lever. A 120sec time-out followed each infusion during which responses on the active lever did not lead to further infusions. Infusions were delivered over a 30sec interval, previously shown to support strong nicotine self-administration and was suggested as the best model of nicotine pharmacokinetics (Sorge & Clarke, 2009). Depression of the left (inactive) lever had no programmed consequence. Responses on both levers were recorded.

Surgery

All animals were implanted with chronically indwelling intrajugular catheters. Briefly, animals were placed under deep anaesthesia using Ketamine (90mg/kg; Phoenix Pharm Distributors LTD, New Zealand) and Xylazine (9mg/kg; Phoenix Pharm Distributors LTD, New Zealand) injected intraperitonealy (IP) at 1.36ml/kg. A small opening in the right chest wall was made to expose the jugular vein and another opening was made on the skull. The vein was isolated and a silastic catheter threaded subcutaneously (SC) from the skull into a small incision in the jugular. The catheter was secured, projecting four centimetres into the jugular vein and tied off above the incision. The externalised portion of the catheter, a sawn off and blunted 22g needle, was mounted to the skull with dental acrylic bonded to four anchoring screws (Centrostyle, Italy, Ref.00395) fixed into the skull. A larger screw was set in place on the skullcap to provide an anchor for the operant chamber spring lead. The chest wall incision was closed using superglue and Teramycin powder (Pfizer Animal Health, Australia) was applied to both surgery sites. To aid recovery, 6ml of warm Hartmann's solution was administered (SC) either side of the rat.

Animals were placed in their home cages to recover and pre- and post-surgery (24hr and 48hr), animals were administered the anti-inflammatory Carprofen (5mg/ml, SC; Norbrook NZ LTD, New Zealand). During the recovery period, animals received 0.2ml of daily

experimenter-administered intravenous (IV) infusion of heparinised (30i.u/ml) 0.9% saline solution containing penicillin G potassium (100,000i.u/ml). During self-administration testing, this penicillin solution was administered before and after the self-administration sessions. Once a week, when animals did not engage in self-administration testing (Sundays), 0.15ml of sodium pentobarbitone (50mg/ml, IV; PROVET, New Zealand) was administered to test catheter patency, as has been described in previous reports (Corrigall, 1992).

Drugs

The TPM was purchased from Labstat International (Ontario, Canada) and prepared as previously described (Ambrose *et al.*, 2007). The nicotine content in the TPM was quantified (ESR), and diluted with sterile 0.9% saline so that nicotine levels were equivalent to 30.0µg/kg/inf. The pH was adjusted to 7.0 with NaOH when necessary. The final treatment solution contained 80ug/ml of nicotine and daily TPM aliquots were frozen prior to use. (-)-Nicotine hydrogen tartrate salt (Sigma-Aldrich, New Zealand) was dissolved into sterile 0.9% saline. The pH of the nicotine solution was adjusted to 7.0 using NaOH, necessary for self-administration testing (Corrigall & Coen, 1991) and separated into aliquots containing 1mg/ml nicotine and frozen. Norharmane hydrochloride (Sigma-Aldrich, New Zealand) was added to nicotine aliquots before freezing at a range of concentrations (0.0, 0.013, 0.083, 0.208mg/ml).

Aliquots were thawed at 4 ° Celsius prior to self-administration sessions daily and diluted as required to the appropriate treatment dose. Remaining solution was disposed of at the end of each session.

Harmane (Sigma-Aldrich, New Zealand) was dissolved in 0.5% ethanol and then suspended in 1% Tween-80 and administered IP at a volume of 1ml/kg 1hr prior to

self-administration treatment.

An easily dissolved form of harman was not available at the commencement of the experiment making it impossible to combine harman solution with the nicotine aliquots. Route of administration, and pretreatment time were selected based on previous micro dialysis work (Baum *et al.*, 1996) which demonstrated that 60min following IP administration, harman produced increased extracellular DA levels in the NAc, a brain region implicated in nicotine self-administration and the behavioural properties of other drugs of abuse (Pontieri *et al.*, 1996).

Experiment 1

Daily self-administration sessions (starting 7:00am, 2hr duration, Monday - Friday) began with the rats being weighed and flushed with penicillin solution. Rats were then transported to the testing room and placed into their designated chambers equipped with a syringe corresponding to their treatment group; nicotine (30.0μg/kg/infusion), TPM, or vehicle. The steel tip of the catheter was uncovered, and rats were attached to the infusion apparatus in the chambers and delivered an initial 0.1ml drug treatment to clear the catheter contents.

Using similar protocols for the acquisition of nicotine self-administration as previous reports (Corrigall, 1992), animals were first placed on a single response fixed ratio (FR1) schedule of reinforcement. Once 10 days of FR1 responding were completed, rats were moved to an FR2 schedule for 5 days, and then an FR5 schedule for a minimum of 10 days prior to the commencement of progressive ratio (PR) testing. The experimenter delivered an additional 3 infusions to all animals on the first day of self-administration, and 2 infusions on the second to encourage initial lever responses. Animals were determined to be non-acquired if they

emitted less than an average of four responses over their last four sessions at FR1, and were excluded from the rest of the study. Unlike the procedures described by Corrigall (1992) animals did not learn any food reinforced operant responses prior to the commencement of drug self-administration training.

PR testing was conducted over several weeks and consisted of multiple self-administration sessions run under a PR schedule of reinforcement. The PR schedule was selected from previous reports (Sorge & Clarke, 2009) and was based on the equation; $5e^{(0.2 \times Infusion)}-5$ (rounded to the nearest integer), which provided the following increasing response requirements: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, and 50. PR sessions occurred every 3 days, and were separated by self-administration sessions run under an FR5 schedule of reinforcement allowing animals to return to stable baseline FR5 responding between PR test sessions.

Experiment 2

Animals were assigned to one of the following treatment groups: nicotine 30.0µg/kg/infusion + norharman: harman (0.0, 0.4, 2.5µg/kg/infusion: 0.0, 1.6, 10.0µg/kg/infusion) or nicotine 75.0µg/kg/infusion + norharman: harman (0.0, 6.25µg/kg/infusion: 0.0, 10.0µg/kg/infusion). The ratio of norharman to harman used for each treatment group was selected to best reflect the relative concentrations of each substance found in mainstream cigarette brands where the proportion of norharman was roughly 2.5 times greater than the concentration of harman (2.19ug/cigarette, 8.52ug/cigarette respectively; Herraiz & Chaparro, 2005). The harman dose was scaled up to account for an expectation that rats which have acquired nicotine self-administration will take on average 10 infusions per session, as is commonly reported (Donny *et al.*, 1995). Animals were pre-treated with

harman/vehicle in their home cages 1 hr before all self-administration sessions.

The acquisition phase for experiment 2 was similar to that of experiment 1, but with some changes to the response criteria for progression to the next reinforcement training schedule. There is evidence that harman and norharman's effects may be related to a gradual accumulation of these substances in tissue (Rommelspacher, 2002). To account for this, animals were allowed up to an additional 10 days at FR1 (20 days in total) to demonstrate stable responding. In addition to maintaining stable response rates prior to progression to a higher FR, animals were also required to demonstrate 3 consecutive days with an average minimum of 4 responses per session FR1, 8 responses at FR2 and 20 responses at FR5 before they could progress to PR testing. Animals remained on their training schedule until these criteria were met, or responding declined substantially. PR testing sessions were conducted in the same manner as in experiment 1.

Data Analysis

For both experiment 1 and 2 only the last 10 days of FR1, 3 days of FR2 and 10 days of FR5 for each rat before progressing on to the next phase of the experiment were analysed. Animals that did not complete the FR5 portion of the acquisition phase (n = 27) due to either a loss of catheter patency, or insufficient active lever responding, and were excluded from analysis. Of those that completed the FR5 phase of acquisition, animals (n = 24) that did not complete a minimum of three PR sessions were excluded from the PR analyses.

All treatment groups that were part of experiment 1 were analysed together. The animals in experiment 2 were split into three different treatment group comparisons to assess the effects of:

1) Nicotine dose (0.0, 30.0, 75.0μg/kg/infusion)

2) Norharman and harman (0.0 / 0.0, 0.4 / 1.6, 2.5μg/kg/infusion norharman / 10.0μg/kg harman) combined with the low nicotine dose (0.0, 30.0μg/kg/infusion)

3) Norharman and harman (0.0 / 0.0, 6.25μg/kg/infusion norharman / 10.0μg/kg harman) combined with the high nicotine dose (0.0, 75.0μg/kg/infusion).

Three-way repeated measures ANOVA were conducted separately for each FR schedule of reinforcement (FR1, FR2, FR5) with active vs. inactive lever and session number as the within subject factors, and self-administration treatment as the between subject factor. Two-way repeated measures ANOVA with session number as the within subject factor, and treatment as the between subject factor, were used to analyses the last three PR breakpoints produced by each rat for each treatment. In the event a significant main effect of treatment was found in any of the analyses Fisher's least squared differences post-hoc tests were conducted to determine treatment specific differences. An alpha level of $\alpha = 0.05$ was used for all significance testing.

Results

Experiment 1: Reinforcing efficacy and motivation to respond for tobacco particulate matter compared to nicotine alone

Acquisition of fixed ratio responding

Acquisition of FR responding for 30.0μg/kg/infusion nicotine (n = 8), TPM (containing 30.0μg/kg/infusion nicotine; n = 11) and 0.9% saline (n = 10) is presented in *Fig 1.1*. During FR1 training, average daily responding increased with a main effect of day ($F(5, 118) = 6.513$, $p < 0.001$). A non-significant main effect of lever ($F(1, 26) = 1.078$, NS) suggested increases

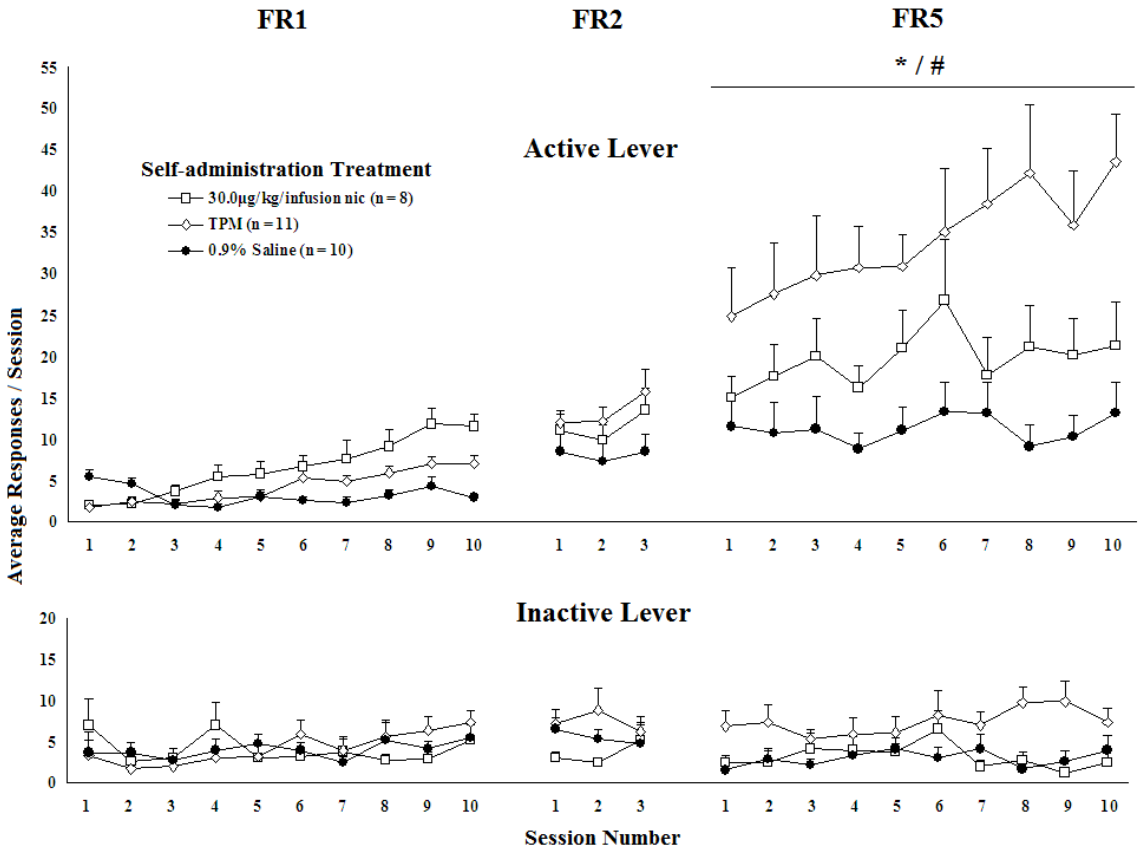

Figure 1.1. Intravenous self-administration of nicotine (nic: 30.0µg/kg/infusion), TPM, or 0.9% saline for the last 10 sessions of FR1, 3 of FR2 and 10 of FR5 completed. Data points represent group mean (+ S.E.M) number of responses during 2hr sessions on the active/inactive lever. Asterisks (*****) indicate a significant difference between TPM and nic treatments ($p < 0.01$). Hash symbols (**#**) indicate a significant difference between TPM and 0.9% saline treatments ($p < 0.001$).

were distributed across both the active and inactive lever. Treatment had no significant effect at FR1 ($F(2, 26) = 1.580$, NS) and there were no significant treatment X lever ($F(2, 26) = 3.282$, NS) or treatment X day ($9, 118) = 1.847$, NS) interactions.

Animals selectively increased active lever responding when the schedule was increased to FR2, as indicated by a main effect of lever ($F(1, 26) = 22.415$, $p < 0.001$). A non-significant main effect of day ($F(2, 52) = 1.123$, NS) and day X treatment interaction ($F(4, 52) = 0.917$, NS) indicated stable responding for all treatment groups. There was no evidence for any differences in active lever responding between treatment groups, as there was no effect of

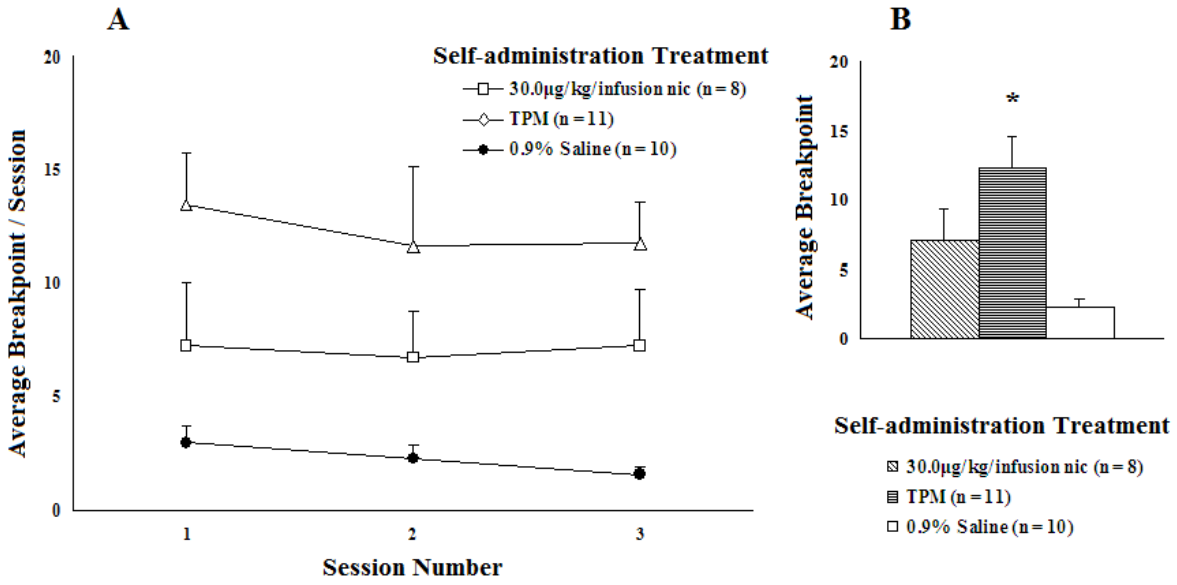

Figure 1.2. Intravenous self-administration of nicotine (nic: 30.0µg/kg/infusion), TPM, or 0.9% saline for the last 3 sessions of PR completed. Data points (**A**) represent group mean (+ S.E.M) breakpoints reached per session. Bars (**B**) represent group mean (+ S.E.M) breakpoints reached across all sessions. Asterisks (*****) indicate treatments that produces significantly greater breakpoints than 0.9% saline ($p < 0.01$).

treatment ($F(2, 26) = 1.944$, NS) or lever X treatment interaction ($F(2, 26) = 1.804$, NS).

Preference for the active lever continued at FR5, indicated by a main effect of lever ($F(1, 26) = 90.886$, $p < 0.001$) which was stable for all treatments during FR5 training due to a non-significant effect of day ($F(3, 66) = 1.660$, NS) and day X treatment interaction ($F(5, 66) = 0.952$, NS). A significant effect of treatment ($F(2, 26) = 11.133$, $p < 0.001$), lever X treatment interaction ($F(2, 26) = 9.595$, $p < 0.01$) was identified at FR5. Post-hoc tests revealed that the TPM solution supported significantly greater FR5 responding than 30.0µg/kg/infusion nicotine ($p < 0.01$) and 0.9% saline ($p < 0.001$).

Progressive ratio testing

PR breakpoints (BP) for TPM (n = 11), 30.0µg/kg/infusion nicotine (n = 8) and 0.9% saline (n = 10) are presented in *Fig 1.2*. No main effect of day ($F(2, 52) = 0.764$, NS) or day X

treatment interaction ($F(4, 52) = 0.203$, NS), indicated that BP remained stable for the duration of test sessions for all treatments. A main effect of treatment ($F(2, 26) = 7.552$, $p < 0.01$) indicated that there were differences in the BP produced by the different self-administration treatments. The post-hoc analysis revealed that the BP achieved by rats receiving TPM was significantly greater than those receiving 0.9% saline ($p < 0.01$). However, 30.0µg/kg/infusion nicotine did not significantly differ from either treatment.

Experiment 2: Effects of norharman and harman on the reinforcing efficacy and motivation to respond for nicotine

Fixed ratio responding for different doses of nicotine

Acquisition of nicotine (0.0, 30.0, 75.0µg/kg/infusion; n = 10, n = 14, n = 6) self-administration on FR1, FR2 and FR5 schedules of reinforcement are presented in *Fig 2.1*. During the FR1 phase, a main effect of day ($F(4, 103) = 7.957$, $p < 0.001$) and lever ($F(1, 27) = 14.980$, $p < 0.01$) suggested average daily responding increased during FR1 training with a preference for the active lever. Acquisition was similar for all treatments, as there was a non-significant day X treatment interaction ($F(8, 103) = 1.819$, NS). However, a main effect of treatment ($F(2, 27) = 4.496$, $p < 0.05$), and lever X treatment interaction ($F(2, 27) = 6.397$, $p < 0.01$) suggested treatment group differences in active lever responding. Post-hoc tests showed that responding was greater for the 75.0µg/kg/infusion nicotine condition, than for the 0.9% saline condition ($p < 0.01$).

There was no main effect of day ($F(2, 54) = 0.988$, NS) or day X treatment interaction ($F(4, 54) = 0.877$, NS), indicating stable responding at FR2. A main effect of lever ($F(1, 27) = 60.907$, $p < 0.001$), treatment ($F(2, 27) = 5.463$, $p < 0.05$) and lever X treatment interaction

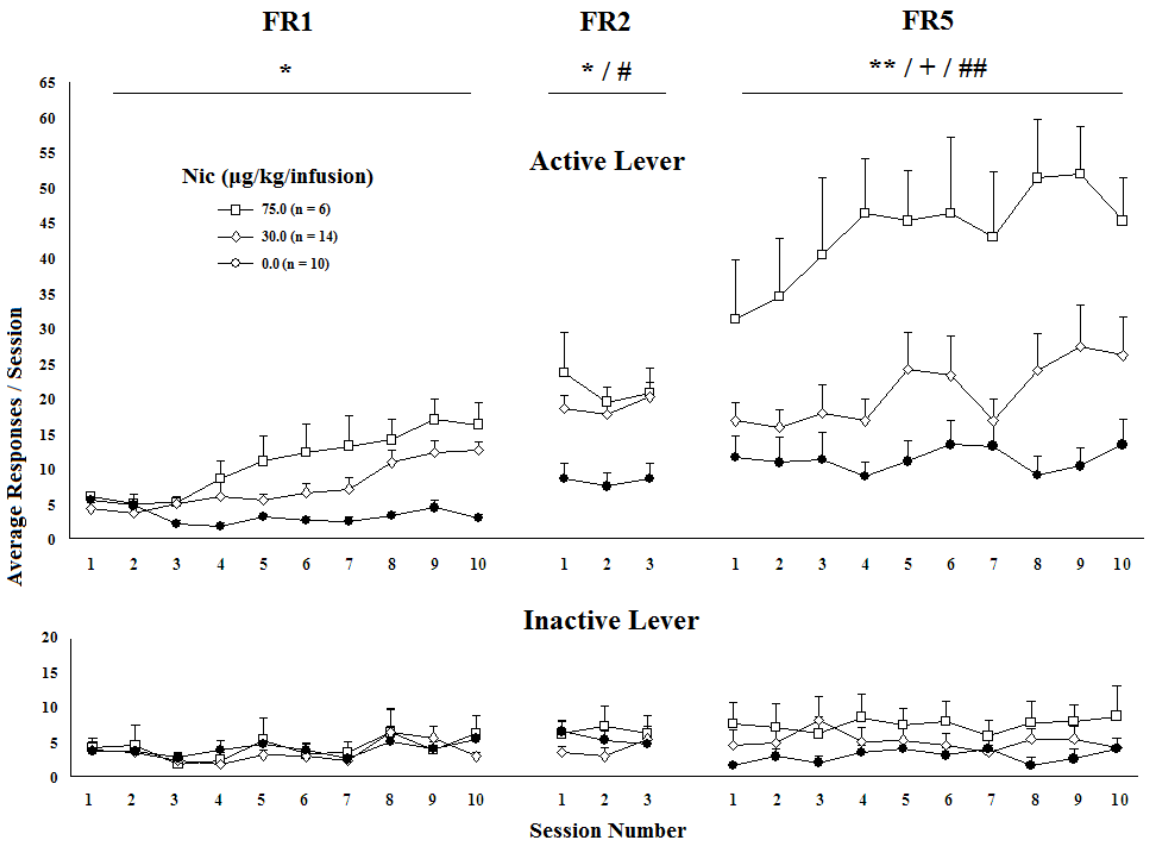

Figure 2.1. Intravenous self-administration of nicotine (nic: 0.0, 30.0, 75.0µg/kg/infusion) for the last 10 sessions of FR1, 3 of FR2 and 10 of FR5 completed. Data points represent group mean (+ S.E.M) number of responses during 2hr sessions on the active/inactive lever. Asterisks (*) indicate a significant difference between 0.0 and 75.0µg/kg/infusion nic (*$p < 0.01$, **$p < 0.001$). Hash symbols (#) indicate a significant difference between 0.0 and 30.0µg/kg/infusion nic (#$p < 0.05$, ##$p < 0.001$). Plus signs (+) indicate a significant difference between 30.0 and 75.0µg/kg/infusion nic ($p < 0.05$).

($F(2, 27) = 10.187$, $p < 0.01$) suggested greater active lever responding, which was more pronounced for some treatments. Post-hoc tests revealed that FR2 responding for both 30.0 and 75.0µg/kg/infusion nicotine was significantly greater than 0.9% saline ($p < 0.05$; $p < 0.01$).

Main effects of lever ($F(1, 27) = 48.280$, $p < 0.001$), treatment ($F(2, 27) = 16.874$, $p < 0.001$) and a lever X treatment interaction ($F(2, 27) = 6.713$, $p < 0.01$) at FR5 indicated that the differences in active lever responding persisted. Post-hoc tests identified that

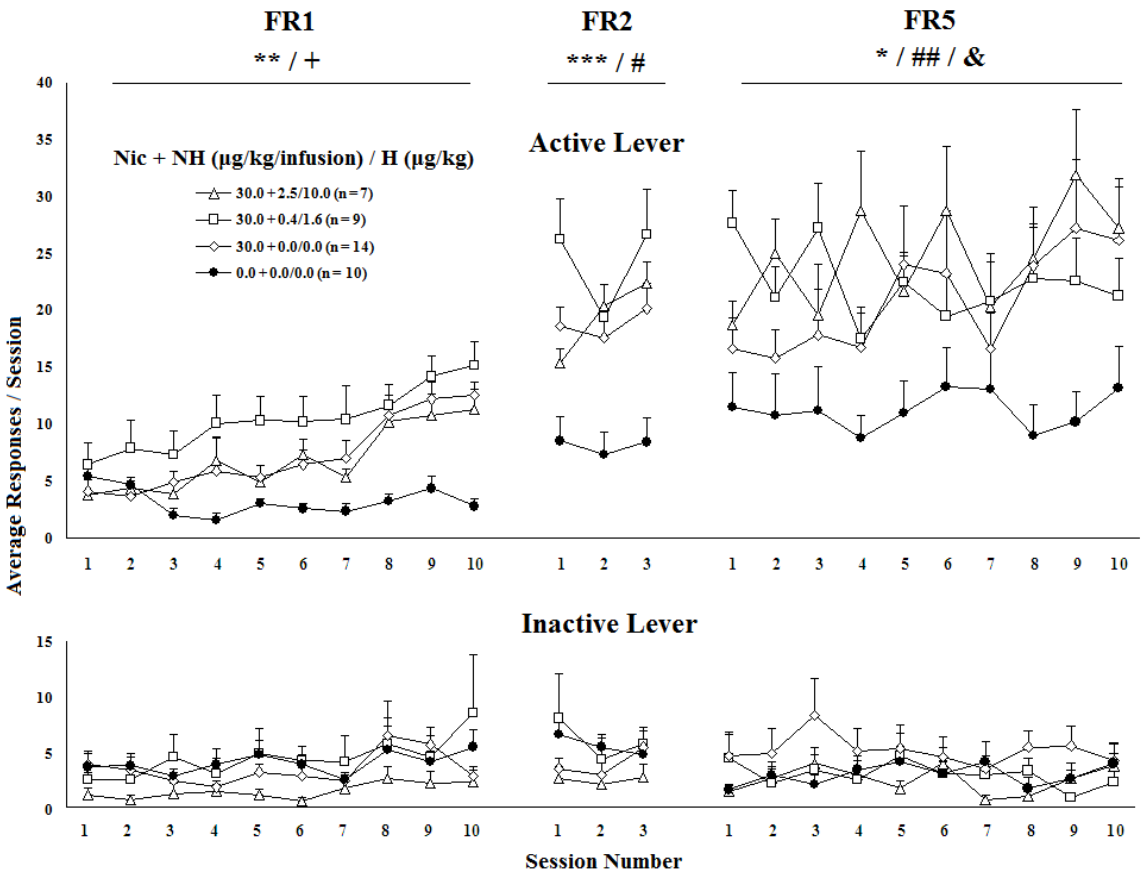

Figure 2.2. Intravenous self-administration of nicotine (nic: 0.0, 30.0µg/kg/infusion) + norharman (NH: 0.0, 0.4, 2.5µg/kg/infusion) for rats pretreated with harman (H: 0.0, 1.6, 10.0µg/kg) for the last 10 sessions of FR1, 3 of FR2 and 10 of FR5 completed. Data points represent group mean (+ S.E.M) number of responses during 2hr sessions on the active/inactive lever. Asterisks (*****) indicate a significant difference between 0.0 + 0.0 / 0.0 and 30.0µg/kg/infusion nic + 0.4µg/kg/infusion NH / 1.6µg/kg H treatments (*****$p < 0.05$, ******$p < 0.01$, *******$p < 0.001$). Hash symbols (#) indicate a significant difference between 0.0 + 0.0 / 0.0 and 30.0µg/kg/infusion nic + 0.0µg/kg/infusion NH / 0.0µg/kg H treatments (#$p < 0.05$, ##$p < 0.01$). Plus signs (+) indicate a significant difference between 30.0 + 0.4 / 1.6 and 30.0µg/kg/infusion nic + 2.5µg/kg/infusion NH / 10.0µg/kg H treatments ($p < 0.05$). Ampersands (**&**) indicate a significant difference between 0.0 + 0.0 / 0.0 and 30.0µg/kg/infusion nic + 2.5µg/kg/infusion NH / 10.0µg/kg H treatments ($p < 0.05$).

75.0µg/kg/infusion nicotine supported greater responding than 30.0µg/kg/infusion nicotine ($p < 0.001$) and 0.9% saline ($p < 0.001$), where 30.0µg/kg/infusion nicotine responding was also significantly greater than 0.9% saline ($p < 0.05$). At FR5, all treatments also demonstrated a gradual increase in responding as shown by a main effect of day ($F(5, 124) = 2.449$, $p < 0.05$), but no day X treatment interaction ($F(9, 124) = 1.055$, NS).

Fixed ratio responding for low dose nicotine combined with norharman and harman

Acquisition of 0.9% saline (n = 10) or 30.0μg/kg/infusion nicotine + norharman (0.0, 0.4, 2.5μg/kg/infusion; n = 14, n = 9, n = 7) self-administration under FR schedules of reinforcement following pretreatment with harman (0.0, 1.6, 10.0μg/kg) are presented in *Fig 2.2*. Main effects of lever ($F(1, 36) = 27.986$, $p < 0.001$) and day ($F(4, 152) = 8.485$, $p < 0.001$) indicate preferential responding on the active lever which increased during FR1 training. A non-significant day X treatment interaction ($F(13, 152) = 1,179$, NS) indicated that all treatment groups increased responding, however a main effect of treatment ($F(3, 36) = 3.858$, $p < 0.05$) and lever X treatment interaction ($F(3, 36) = 5.172$, $p < 0.01$) suggested differences in overall active lever responses. Post hoc tests revealed that at FR1, rats treated with 30.0μg/kg/infusion nicotine + 0.4μg/kg/infusion norharman / 1.6μg/kg harman exhibited a higher number of responses than those treated with 30.0μg/kg/infusion nicotine + 2.5μg/kg/infusion norharman / 10.0μg/kg harman ($p < 0.05$) or 0.9% saline ($p < 0.01$).

Responding continued to increase for all treatments when moved to FR2 as shown by a main effect of day ($F(2, 72) = 3.159$, $p < 0.05$) and non-significant day X treatment interaction ($F(6, 72) = 1.872$, NS). Similarly, a main effect of lever ($F(1, 36) = 145.728$, $p < 0.001$), treatment ($F(3, 36) = 5.866$, $p < 0.01$) and lever X treatment interaction ($F(3, 36) = 11.296$, $p < 0.001$) persisted at FR2. Post-hoc tests identified the effect of treatment to be the result of greater responding produced by 30.0μg/kg/infusion nicotine + 0.0μg/kg/infusion norharman / 0.0μg/kg harman and 30.0μg/kg/infusion nicotine + 0.4μg/kg/infusion norharman / 1.6μg/kg harman relative to 0.9% saline ($p < 0.05$; $p < 0.001$).

At FR5, there was no effect of day ($F(5, 167) = 1.360$, NS) or day X treatment interaction ($F(14, 167) = 1.650$, NS), indicating stable levels of responding for all treatment groups. Main effects of lever ($F(1, 36) = 67.200$, $p < 0.001$) and treatment ($F(3, 36) = 3.308$, p

< 0.05) indicated preferential responding for the active lever and treatment differences, however a non-significant lever X treatment interaction ($F(14, 167) = 1.650$, NS) suggested these differences were not active lever exclusive. Post-hoc analysis revealed that 30.0µg/kg/infusion nicotine combined with 0.0, 0.4, 2.5µg/kg/infusion norharman / 0.0, 1.6, 10.0µg/kg harman all produced significantly greater responding than 0.9% saline ($p < 0.01$; $p < 0.05$; $p < 0.05$) but were not significantly different from each other.

Fixed ratio responding for high dose nicotine combined with norharman and harman

Fig 2.3 presents acquisition of FR responding for 0.9% saline (n = 10) or 75.0µg/kg/infusion nicotine + norharman (0.0, 6.25µg/kg/infusion; n = 6, n = 10) following daily pretreatment with harman (0.0, 10.0µg/kg). Responding increased during FR1 training in a treatment dependent manner with a main effect of day ($F(4, 98) = 9.369$, $p < 0.001$), treatment ($F(2, 23) = 4.860$, $p < 0.05$) and day X treatment interaction ($F(9, 98) = 2.315$, $p < 0.05$). Post-hoc analysis indicated that 75.0µg/kg/infusion nicotine + 0.0µg/kg/infusion norharman / 0.0µg/kg harman treatment led to significantly greater responding than 0.9% saline ($p < 0.01$). A main effect of lever ($F(1, 23) = 8.793$, $p < 0.01$) and lever X treatment interaction ($F(2, 23) = 5.567$, $p < 0.05$) suggested that responding was directed towards the active lever, but there were treatment-produced differences in the level of active lever selectivity.

Responding was stable for all treatments at FR2 with no effect of day ($F(2, 46) = 1.850$, NS) or day X treatment interaction ($F(4, 46) = 1.351$, NS). Main effects of lever ($F(1, 23) = 52.340$, $p < 0.001$), treatment ($F(2, 23) = 6.772$, $p < 0.01$), and a lever X treatment interaction ($F(2, 23) = 9.335$, $p < 0.01$) indicated a clear preference for the active lever. Post-hoc tests revealed greater responding for 75.0µg/kg/infusion nicotine + 0.0 / 0.0, 6.25µg/kg/infusion

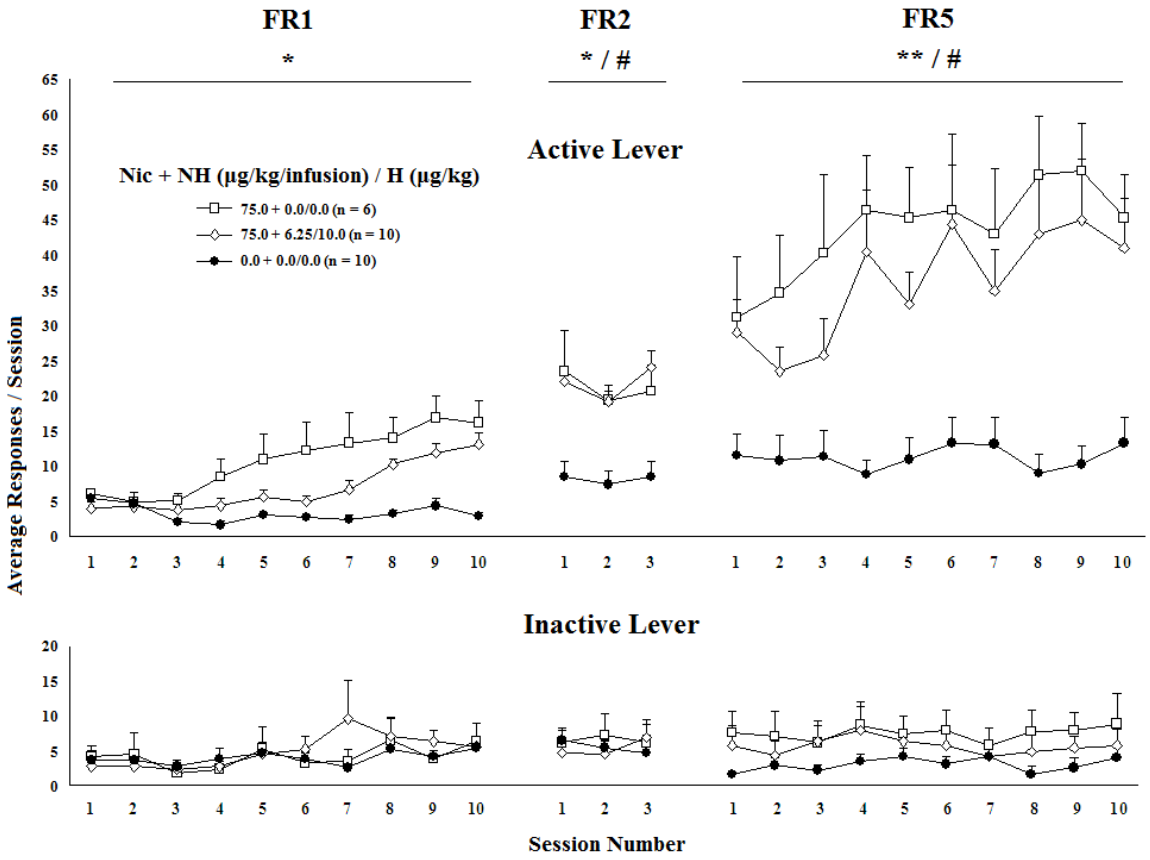

Figure 2.3. Intravenous self-administration of nicotine (nic: 0.0, 75.0µg/kg/infusion) + norharman (NH: 0.0, 6.25µg/kg/infusion) for rats pretreated with harman (H: 0.0, 10.0µg/kg) for the last 10 sessions of FR1, 3 of FR2 and 10 of FR5 completed. Data points represent group mean (+ S.E.M) number of responses during 2hr sessions on the active/inactive lever. Asterisks (*) indicate a significant difference between 0.0 + 0.0 / 0.0 and 75.0µg/kg/infusion nic + 0.0µg/kg/infusion NH / 0.0µg/kg H treatments (*$p < 0.01$, **$p < 0.001$). Hash symbols (#) indicate a significant difference between 0.0 + 0.0 / 0.0 and 75.0µg/kg/infusion nic + 6.25µg/kg/infusion NH / 10.0µg/kg H treatments ($p < 0.01$).

norharman / 10.0µg/kg harman than for 0.9% saline ($p < 0.01$; $p < 0.01$).

A main effect of day ($F(5, 104) = 3.376$, $p < 0.01$) and a non-significant day X treatment interaction ($F(9, 104) = 1.254$, NS) at FR5 indicated responding gradually increased when the response requirement was increased in a treatment independent manner. Preference for the active lever continued at FR5, with a main effect of lever ($F(1, 23) = 86.590$, $p < 0.001$). A main effect of treatment ($F(2, 23) = 11.277$, $p < 0.001$) and lever X treatment interaction ($F(2, 23) = 10.625$, $p < 0.01$) indicated that there were differences in active lever responding

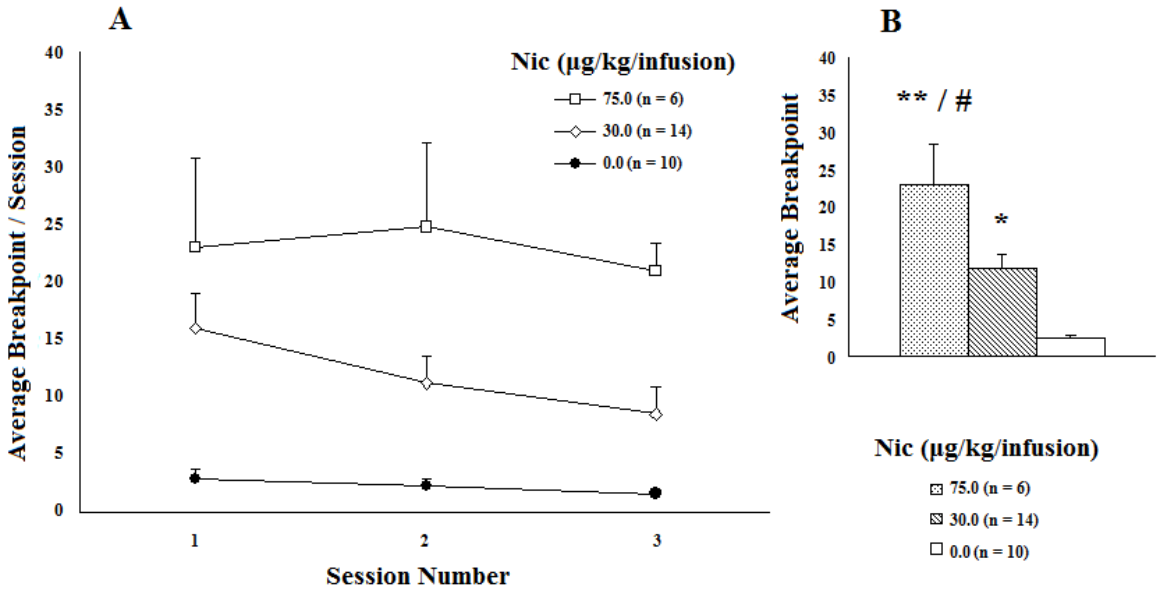

Figure 2.4. Intravenous self-administration of nicotine (nic: 0.0, 30.0, 75.0µg/kg/infusion) for the last 3 sessions of PR completed. Data points (**A**) represent group mean (+ S.E.M) breakpoints reached per session. Bars (**B**) represent group mean (+ S.E.M) breakpoints reached across all sessions. Asterisks (*****) indicate treatments that produces significantly greater breakpoints than 0.9% saline (*****$p < 0.05$, ******$p < 0.001$). Hash symbols (#) indicate treatments that produce significantly greater breakpoints than 30.0µg/kg/infusion nic ($p < 0.05$).

between treatment groups. This was confirmed by post-hoc analysis showing that 75.0µg/kg/infusion nicotine + 0.0, 6.25µg/kg/infusion norharman / 0.0, 10.0µg/kg harman produced greater responding than 0.9% saline ($p < 0.001$; $p < 0.01$) similar to FR2.

Progressive ratio testing for nicotine combined with norharman and harman

Presented in *Fig 2.4*, a main effect of treatment was found when comparing 30.0 (n = 4), 75.0µg/kg/infusion nicotine (n = 5) and 0.9% saline (n = 10; $F(2, 16) = 17.517$, $p < 0.001$). The 75.0µg/kg/infusion nicotine treatment consistently produced significantly greater BP than the 30.0µg/kg/infusion nicotine ($p < 0.05$) and 0.9% saline ($p < 0.01$) conditions. The 30.0µg/kg/infusion nicotine condition also produced greater average BP than 0.9% saline ($p < 0.05$). BP remained stable throughout testing for all treatments, evidenced by no main effect

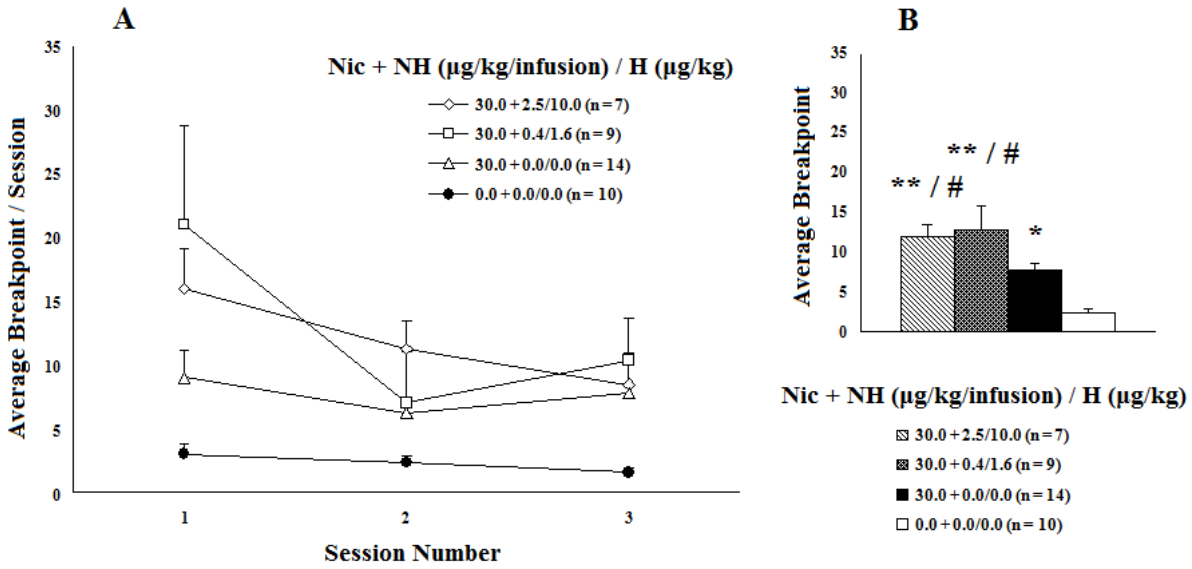

Figure 2.5. Intravenous self-administration of nicotine (nic: 0.0, 30.0µg/kg/infusion) + norharman (NH: 0.0, 0.4, 2.5µg/kg/infusion) for rats pretreated with harman (H: 0.0, 1.6, 10.0µg/kg) for the last 3 sessions of PR completed. Data points (**A**) represent group mean (+ S.E.M) breakpoints reached per session. Bars (**B**) represent group mean (+ S.E.M) breakpoints reached across all sessions. Asterisks (*****) indicate treatments that produces significantly greater breakpoints than 0.9% saline (*****$p < 0.01$, ******$p < 0.001$). Hash symbols (#) indicate treatments that produce significantly greater breakpoints than 30.0µg/kg/infusion nic + 2.5µg/kg/infusion NH / 10.0µg/kg H ($p < 0.05$).

of day ($F(2, 32) = 2.375$, NS) or day X treatment interaction ($F(4, 32) = 0.858$, NS).

Comparing 30.0µg/kg/infusion nicotine combined with 0.0/ 0.0 (n = 4), 0.4 / 1.6 (n = 3) and 2.5µg/kg/infusion norharman / 10.0µg/kg harman (n = 4) and 0.9% saline (n = 10; *Fig 2.5*) revealed a main effect of treatment ($F(3, 17) = 19.291$, $p < 0.001$). Post-hoc tests showed that 30.0µg/kg/infusion nicotine plus 0.0/ 0.0 and 0.4µg/kg/infusion norharman / 1.6µg/kg harman produced significantly greater BP than both the 0.9% saline ($p < 0.001$; $p < 0.001$) and 30.0µg/kg/infusion nicotine plus 2.5µg/kg/infusion norharman / 10.0µg/kg harman ($p < 0.05$; $p < 0.05$) conditions. Additionally, 30.0µg/kg/infusion nicotine plus 2.5µg/kg/infusion norharman / 10.0µg/kg harman also yielded greater BP than 0.9% saline ($p < 0.01$). A main effect of day ($F(2, 29) = 8.987$, $p < 0.01$) and non-significant day X treatment interaction ($F(5,$

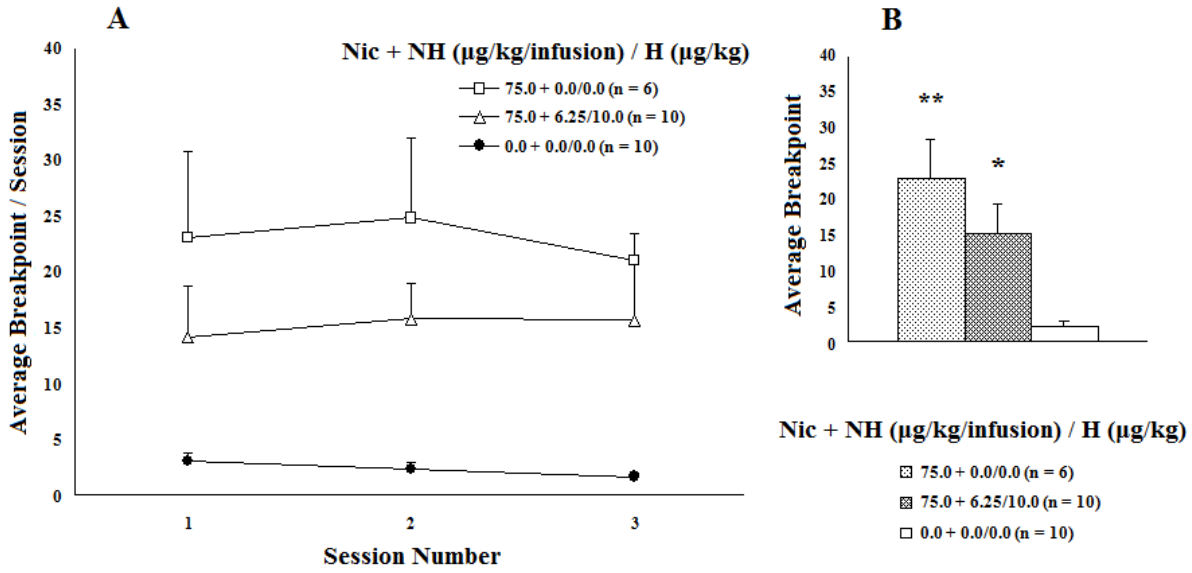

Figure 2.6. Intravenous self-administration of nicotine (nic: 0.0, 75.0µg/kg/infusion) + norharman (NH: 0.0, 6.25µg/kg/infusion) for rats pretreated with harman (H: 0.0, 10.0µg/kg) for the last 3 sessions of PR completed. Data points (**A**) represent group mean (+ S.E.M) breakpoints reached per session. Bars (**B**) represent group mean (+ S.E.M) breakpoints reached across all sessions. Asterisks (*****) indicate treatments that produce significantly greater breakpoints than 0.9% saline (*****$p < 0.01$, ******$p < 0.001$).

29) = 2.330, NS) indicated that BP systematically decreased for all treatments following repeated testing.

In *Fig 2.6*, 75.0µg/kg/infusion nicotine is shown combined with 0.0 / 0.0 (n = 5) or 2.5µg/kg/infusion norharman / 10.0µg/kg harman (n = 6) and 0.9% saline (n = 10) treated rats. There was a main effect of treatment ($F(2, 18) = 12.576$, $p < 0.001$) for PR-produced BP. The 75.0µg/kg/infusion nicotine and 0.0 / 0.0 or 6.25µg/kg/infusion norharman / 10.0µg/kg harman treatments produced significantly greater BP than the 0.9% saline ($p < 0.001$; $p < 0.01$) condition. There were no main effects of day ($F(2, 36) = 0.444$, NS) or day X treatment interaction ($F(4, 36) = 0.363$, NS), indicating that BP remained stable for the duration of testing.

Discussion

The present study is the first to demonstrate that rats will intravenously self-administer an aqueous tobacco smoke extract (TPM). The results revealed that TPM was more rewarding than a matched dose of pure nicotine as shown by increased responding on FR5 and higher breakpoints than nicotine and vehicle control groups. A role for the smoke constituents, harman and norharman, in these effects was also investigated. Low dose harman and norharman combined with low dose nicotine initially produced a small enhancement in FR1 responding, but there was a tendency to reduce FR responding and decrease BP for nicotine at the higher harman and norharman doses.

For experiment 1 animals were trained to intravenously self-administer nicotine, TPM (Ambrose et al., 2007) containing an equivalent volume of nicotine, or vehicle delivered under fixed ratio (FR) schedules. The results showed that nicotine and TPM were both more effective at maintaining self-administration under an FR schedule of reinforcement than vehicle, demonstrating compensatory increases in active lever responding when the FR requirements were increased (Figure 1.1). However, low dose nicotine (30.0μg/kg/inf) did not support robust self-administration (Figure 1.1). The nicotine group did not exhibit responding above control levels on FR1. Responding increased on FR2 relative to the controls, but when the response requirement was increased to FR5, responding dropped and was again no different to controls. These results were comparable to previous nicotine self-administration studies that have shown that when the response requirement is increased; motivation to maintain a similar nicotine intake is low (Clemens *et al.*, 2009; Clemens *et al.*, 2010) or there was failure to observe self-administration altogether (Guillem *et al.*, 2005; 2006). This general pattern extended to the PR tests in the present study, where overall BP were not significantly

higher for nicotine compared to controls (Figure 1.2).

Acquisition of self-administration at FR1 and FR2 did not differ between treatments, suggesting that TPM did not produce an acutely more rewarding experience compared to nicotine at the start of the experiments. However, TPM supported greater responding at FR5 and produced a higher BP in PR tests than the nicotine and vehicle control groups (Figure 1.2), indicating that the rats were more motivated to receive TPM. These results suggest that chronic exposure to TPM might be required in combination with an increased response requirement for the differences between treatment groups to become evident. Interestingly, increasing response requirements are required to identify differences between the motivation to work for intravenous nicotine and placebo when testing human tobacco smokers (Harvey *et al.*, 2004).

Tobacco smoke extract exposure has produced effects on addiction related reward pathways in the brain which are not produced by nicotine alone. For example Small *et al.* (2010) found that tobacco smoke exposure reduced response latencies in an intra-cranial self-stimulation paradigm, whilst nicotine did not. The ability to reduced response latencies is a property of psychostimulant drugs that have been implicated in reinforcement processes (Wise & Bozarth, 1987). Acquisition of intravenous nicotine self-administration and the development of nicotine dependence rely on acute activation of nAChRs and chronic desensitisation and upregulation of these receptors in mesoaccumbens DA circuitry (Brennan et al., 2010). Since TPM has upregulated nAChRs to a greater extent than an equivalent dose of nicotine (Ambrose et al., 2007), it is possible that upregulated nAChRs could underlie the enhanced behavioural response to TPM observed in the present study. Furthermore, TPM alone exhibits strong MAO inhibitory activity (Lewis and Truman, ESR unpublished data) and tobacco smoke exposure produces brain MAO inhibition (Fowler *et al.*, 1996a; 1996b;

Sharma & Brody, 2009), whereas nicotine injection does not produce these effects (Fowler *et al.*, 1998). Thus, at least one of these TPM-produced neuroadaptations could account for the observed enhancement of reward.

The purpose of experiment 2 was to determine whether harman and norharman could enhance the rewarding effects of two different concentrations of intravenous nicotine. The results from experiment 2 revealed that the larger nicotine dose (75.0µg/kg/infusion) supported greater self-administration than vehicle at all FR schedules tested, and better FR5 responding than the smaller nicotine dose (30.0µg/kg/infusion) (Figure 2.1). Greater BP were also produced by the higher nicotine dose than both the lower nicotine dose and vehicle, which were also significantly different from each other, indicating nicotine dose dependently increases in reinforcing efficacy.

These nicotine dose-effect findings compare with others, where higher doses of nicotine (60 and 90.0µg/kg/infusion) supported better FR responding (Chaudhri *et al.*, 2007) and also yielded increased BP on PR compared to the lower doses (Donny *et al.*, 1999; Chaudhri *et al.*, 2007). Interestingly, when nicotine paired visual cues were removed the lower doses would only weakly support self-administration if at all (Chaudhri *et al.*, 2007, Sorge & Clarke, 2009). Further, only the very high dose (90.0µg/kg/infusion) maintained self-administration above control levels (Chaudhri *et al.*, 2007). Chaudhri's (2007) results suggest that only high dose nicotine self-administration is maintained by primary reinforcing properties.

The high nicotine dose does not represent levels found in tobacco smoke. The most representative nicotine dose of a 'puff' on a cigarette was purported to be 3.0µg/kg/infusion, where the 30.0µg/kg/infusion dose used in the present study was at the maximal end of the smoke exposure range (Sorge & Clarke, 2009). Therefore the 75.0µg/kg/infusion dose served as a 'positive control' and demonstrated that when the nicotine dose is high enough, nicotine

more closely resembles other reinforcing psychostimulants in self-administration tests. Overall responding for the 30.0μg/kg/infusion (same as nicotine content of TPM) was weak, supporting the idea that nicotine cannot fully account for the reinforcing effects of TPM.

Overall levels of self-administration observed in this study were lower than what is frequently reported for nicotine, particularly at the 30.0μg/kg infusion dose. Studies using similar protocols to this report normally find rats will consistently respond for approximately 10 infusions of nicotine (Donny *et al.*, 1995). However, similar levels of responding to those in this report have been reported elsewhere (Shoaib *et al.*, 1997). There are multiple methodological features which might account for the less than typical response observed in the present study. Animals in this study were not initially trained to respond for a food reinforcer as has been previously recommended (Corrigall, 1992). The only visual stimulus presented during presentation of a nicotine infusion consisted of a key light; however previous reports have turned off the house lighting during the timeout period, which facilitated the acquisition of nicotine self-administration (Caggiula, 2002). Further, the present self-administration sessions were conducted during the light cycle, whereas it has been reported that rodents trained during the dark cycle tended to perform better (Paterson *et al.*, 2010). Despite these methodological differences and the low overall rate of active lever responding, nicotine still maintained significantly more responding than vehicle.

The last major methodological difference which may account for lower rates of nicotine self-administration in this study is the longer 30sec infusion time used. Traditionally nicotine infusions are delivered over 1sec, an infusion time thought to best represent the rapid bolus of nicotine delivered to the brain following the puff of a cigarette (Rose *et al.*, 1999). This view has been supported by previous work which found that a 1sec infusion of nicotine better supports more robust self-administration responding, than nicotine delivered over longer

durations (Valentine *et al.*, 1997). However, an infusion time of 30sec has recently been argued to better reflect nicotine pharmacokinetics during cigarette smoking (Sorge & Clarke, 2009). Sorge and Clarke (2009) looked at the differences produced by the change in infusion time and found 30s infusions with a 120s timeout supported greater active lever responding than 3s infusion on both FR and PR schedules of reinforcement. They also found rats demonstrated a preference for 30sec infusions of nicotine compared to 3sec infusions. Increased responding under a 30sec infusion time found by Sorge & Clarke (2009) make it unlikely the infusion time chosen for this study contributed towards the lower levels of responding.

Experiment 2 revealed that the treatment group that received high dose harman and norharman and high dose nicotine (75.0µg/kg/infusion) did not exhibit much change in behaviour when compared to the nicotine only group (75.0µg/kg/infusion) (Figure 2.3). Initially on FR1, the harman and norharman group exhibited lower levels of responding than the nicotine only group, although these differences disappeared when the response requirement was increased. There were no differences in BP on PR tests (Figures 2.6).

When harman and norharman were combined with low dose nicotine (30.0µg/kg/infusion) a similar suppression of responding at lower FR schedules was observed for the highest dose of harman and norharman (Figure 2.2). This difference vanished once the response schedule was increased to FR5, with all treatments supporting greater self-administration than vehicle. All treatments also produced greater BP than vehicle, however, the highest dose of harman and norharman combined with nicotine supported lower BP than the other groups (other groups (Figure 2.5). These results suggest that harman and norharman might reduce the reinforcing efficacy of nicotine.

The inability of harman and norharman to increase the reinforcing efficacy of

intravenous nicotine suggests they are not responsible for the greater BP produced by TPM treatment in experiment 1. In fact, the results of experiment 2 suggest that norharman and harman may reduce the reinforcing efficacy of nicotine. These findings are unexpected as MAO-I treatment has previously been shown to enhance intravenous self-administration of nicotine (Guillem *et al.*, 2005; 2006). These inconsistencies are likely the result of the type of MAO-I used (tranylcypromine and phenelzine), and doses selected for treatment. These MAO-I have effects additional to their ability to inhibit MAO, and potently increase concentrations of catecholamines such as DA and 5-HT similar to other drugs of abuse (Baker *et al.*, 1992). The dose of norharman used by Guillem *et al.* (2006) that enhanced BP for nicotine self-administration was also substantially larger than what a cigarette smoker might be exposed to.

The finding that harman and norharman reduce the reinforcing efficacy of the lowest dose of nicotine tested however was contrary to the hypothesis of this study. Both harman and norharman produce an increase in synaptic DA in the NAc (Baum *et al.*, 1995; 1996), which would be expected to support or enhance self-administration behaviour (Pontieri *et al.*, 1996; Di Chiara, 2000). However, harman and norharman have effects on catecholamines other than DA and these effects could inhibit behaviour. For example, harman stimulated 5-HT activity at low concentrations (Baum *et al.*, 1996) and increased synaptic levels of 5-HT in the NAc inhibits the firing of DA neurons located in the VTA, believed to be nicotine's primary site of action (Corrigall, 1999).

Suppression of the reinforcing properties of nicotine was most pronounced at the lowest dose of nicotine combined with the highest dose of harman and norharman (Figure 2.5). Interestingly, this treatment condition received the highest dose of harman (10μg/kg). Harman potently inhibits MAO-A, without actions on MAO-B (Herraiz & Chapparo, 2005). Previous

studies have shown that inhibition of MAO-A, but not MAO-B, produced changes in nicotine reinforced self-administration (Guillem *et al.*, 2006). While both forms of MAO are known to inhibit DA (Berlin & Anthenelli, 2001), MAO-A is preferentially responsible for the degradation of 5-HT (Celada & Artigas, 1993). As a result, the suppression of nicotine reinforcing efficacy might be due to a greater increase in synaptic 5-HT relative to DA.

Increases in 5-HT activity have been associated with inhibition of cocaine self-administration. Animals trained to self-administer cocaine on an FR schedule of reinforcement made fewer responses when their daily diet contained L-tryptophan, a 5-HT synthesis precursor that increases CNS levels of 5-HT (Carroll *et al.*, 1990). When rats were tested on a PR schedule of reinforcement with cocaine as a reinforcer, BP achieved following lesioning of 5-HT neurons using 5,7-DHT were markedly increased (Loh & Roberts, 1990). Further, rats pretreated with 5-HT agonists showed reduced self-administration of cocaine, an effect that was more pronounced at low unit doses, and high unit costs (Peltier & Schenk, 1993). These findings suggest that 5-HT primarily plays a role in the inhibitory modulation of cocaine's reinforcing efficacy.

The TPM treatment used in this study is derived from tobacco smoke which contains harman and norharman (quantified by ESR; Christchurch, New Zealand). However, the concentration of harman and norharman found in the TPM solution does not appear to be sufficient to reduce the reinforcing efficacy of this treatment. There are two likely explanations for this lack of inhibition. First, nAChR upregulation and/or increased stimulation of these receptors might enhance reward, where nicotine is not the only tobacco constituent that acts via nAChRs. For example, nornicotine, anabeseine, anabesine and N-methylanabasine are all smoke constituents able to stimulate DA efflux and desensitise nAChRs *in vitro* (Dwoskin *et al.*, 1995). Similarly, cotinine causes upregulation of nAChRs

(O'Leary *et al.*, 2008). These effects, when combined with the actions of nicotine, might produce enough activation of nAChRs on DA neurons to overcome any inhibition produced by harman and norharman. Alternately, concurrent inhibition of 5-HT by other tobacco constituents (Touiki *et al.*, 2007) could counteract the stimulatory actions of harman and norharman on the serotonergic system. In particular, nAChRs located on 5-HT containing cells projecting to various brain regions could be candidates for such a blockade (Seth *et al.*, 2002).

This study determined the relative reinforcing efficacy of an aqueous tobacco extract, nicotine, and nicotine combined with harman and norharman in rats. TPM possessed significantly greater reinforcing efficacy than nicotine, an effect which may be related to differences in the ability of nicotine and TPM to upregulate nAChRs. However, the hypothesised role for harman and norharman in the differences between TPM and nicotine was not supported. Harman and norharman significantly reduced the reinforcing efficacy of intravenous nicotine at the lowest unit dose of nicotine tested. These β-carbolines are known to have multiple central actions including MAO inhibitory properties and the ability to increase synaptic DA and 5-HT. Increases in 5-HT activity causes a reduction in the reinforcing efficacy of psychostimulants such as cocaine, particularly at low unit doses and high unit costs. Thus, excitatory effects of harman and norharman on the serotonergic system might explain the reduction in nicotine reinforcing efficacy observed. Although the aqueous tobacco extract used in this study contained measurable levels of harman and norharman, the present results indicate that other tobacco constituents must be responsible for the enhanced reinforcing efficacy of TPM.

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