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β -carbolines found in cigarette smoke elevate intracranial self-stimulation thresholds in rats

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Abstract

Identifying novel constituents that contribute to tobacco addiction is essential for developing more effective treatments and informing FDA regulation of tobacco products. While preclinical data indicate that monoamine oxidase (MAO) inhibitors can have abuse liability or potentiate the addiction-related effects of nicotine, most of these studies have used clinical MAO inhibitors (e.g., tranylcypromine) that are not present in cigarette smoke. The primary goal of this study was to evaluate the abuse potential of the β -carbolines harmane, norharmane, and harmine - MAO inhibitors that are found in cigarette smoke - in an intracranial self-stimulation (ICSS) model in rats. A secondary goal was to evaluate the ability of norharmane to influence nicotine's acute effects on ICSS. None of the β -carbolines lowered ICSS thresholds at any dose studied when administered alone, suggesting a lack of abuse liability. Rather, all three β -carbolines produced dose-dependent elevations in ICSS thresholds, indicating aversive/anhedonic effects. Harmane and harmine also elevated ICSS response latencies, suggesting a disruption of motor function, albeit with reduced potency compared to their ICSS threshold-elevating effects. Norharmane (2.5 mg/kg) modestly attenuated the effects of nicotine on ICSS thresholds. Our findings indicate that these β -carbolines produced only aversive/anhedonic effects in an ICSS model when administered alone, and that norharmane unexpectedly attenuated nicotine's acute effects on ICSS. Future work evaluating the addiction-related effects of nicotine combined with these and other MAO inhibitors present in smoke may be useful for understanding the role of MAO inhibitors in tobacco addiction and informing FDA tobacco regulation.

Keywords

Nicotine; β -carbolines; monoamine oxidase inhibitors; non-nicotine tobacco constituents; intracranial self-stimulation; rat

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1. Introduction

Nicotine is the primary addictive constituent in tobacco products, but other tobacco constituents may also contribute to tobacco use (e.g., Brennan et al., 2014; Hoffman and Evans, 2013; LeSage et al., 2018). For example, cigarette smoke contains various compounds (e.g., β -carboline, 2-naphthylamine) that inhibit monoamine oxidase (MAO), an enzyme that degrades monoamines (e.g., dopamine, serotonin), and both isozymes of MAO (MAO-A and MAO-B) are inhibited in smokers (Fowler et al., 1996a; Fowler et al., 1996b; Hogg, 2016). Given the critical role for monoamines in addiction, the ability of cigarette smoke to inhibit MAO and thereby increase monoamine levels has been proposed to contribute to tobacco dependence (Berlin and Anthenelli, 2001; Fowler et al., 2003). Consistent with this prediction, preclinical data indicate that MAO inhibitors can produce addiction-related behavioral or neurobiological effects themselves (Ainsworth et al., 1998; Porsolt et al., 1984; Wu and Zhu, 1999) or potentiate the addiction-related effects of nicotine (e.g., Guillem et al., 2005; Kapelewski et al., 2011; Smith et al., 2016; Smith et al., 2015; Villegier et al., 2007). However, most of these studies have used clinical MAO inhibitors (e.g., tranylcypromine, phenelzine) that are not found in cigarette smoke and can have various pharmacological effects other than MAO inhibition (e.g., direct release of monoamines) that may limit the relevance of these findings to smokers (Baker et al., 1992; Lotfipour et al., 2011; Villegier et al., 2011). Characterizing the abuse liability of MAO inhibitors that are present in smoke is essential for understanding the role of MAO inhibition in tobacco dependence and could lead to better treatments for smoking cessation. Such work could also inform the development of product standards by the Food and Drug Administration Center for Tobacco Products (FDA CTP) aimed at reducing the addictiveness of tobacco products.

The β -carboline norharmane and harmane (also called norharman and harman) are non-selective and MAO-A inhibitors, respectively, that are present in cigarette smoke at considerable concentrations (up to $\approx 6,000$ and $2,500$ ng / cigarette, respectively) (Totsuka et al., 1999; Zhang et al., 2011). Both of these β -carbolines have been proposed to play a key role in the MAO inhibitory properties of cigarette smoke (Herraiz, 2004; Herraiz and Chaparro, 2005). Based on tobacco industry documents, the β -carboline MAO-A inhibitor harmine is also present in cigarette smoke at unspecified concentrations (Philip Morris, 2000). Regardless of its levels in smoke, harmine is a proposed metabolite of harmane (Guan et al., 2001; but see Li et al., 2016), and could contribute to the MAO inhibitory properties of smoke via this mechanism.

Norharmane, harmane, and harmine can produce a range of behavioral effects in preclinical models including cognitive effects, anxiolytic effects, and antidepressant effects (e.g., Abelaira et al., 2013; Aricioglu and Altunbas, 2003; Celikyurt et al., 2013; Fortunato et al., 2010a; Patel et al., 2012). They can also produce addiction-related neurobiological effects, including increased dopamine levels in the nucleus accumbens or striatum (Baum et al., 1995, 1996; Iurlo et al., 2001) or increased firing and burst activity of dopamine neurons in the ventral tegmental area (Arib et al., 2010). However, only limited data are available on their addiction-related behavioral effects. Norharmane was itself reinforcing in an i.v. self-administration model (Arnold et al., 2014), and potentiated the reinforcing effects of

nicotine when combined with the nicotine unit dose (Arnold et al., 2014) or when given as a pretreatment (Guillem et al., 2006). In contrast, another study found that neither harmaline nor norharmaline pretreatment affected ongoing nicotine self-administration (Hall et al., 2014), and a cocktail of behaviorally active constituents including norharmaline, harmaline, acetaldehyde, and minor alkaloids (e.g., nornicotine, anabasine) did not alter nicotine's reinforcing effects (Smith et al., 2015). Although harmaline produced anhedonic effects in a sucrose preference test (Giacobbo et al., 2020), it has not been evaluated in a model of abuse liability *per se*.

Intracranial self-stimulation (ICSS) has been useful for studying the addiction-related effects of drugs on brain reinforcement systems (e.g., Negus and Miller, 2014; Wise, 1996). In this assay, animals respond for pulses of electrical stimulation delivered to reinforcement-related brain areas such as the lateral hypothalamus. The minimal or threshold stimulation intensity that maintains responding provides a sensitive measure of the function of brain reinforcement systems. At low to moderate doses, nicotine reduces ICSS thresholds, suggesting an enhancement of the reinforcing effects of the brain stimulation (Caggiula et al., 2009; Harrison et al., 2002; Huston-Lyons and Kornetsky, 1992). More generally, this effect may reflect the potential for nicotine to enhance the reinforcing effects of environmental stimuli (e.g., sensory stimuli, food) (see Caggiula et al., 2009; Chaudhri et al., 2006; Paterson et al., 2008; Wise, 2002), a behavioral mechanism proposed to mediate tobacco dependence (Caggiula et al., 2009; Chaudhri et al., 2006; Paterson, 2009). Importantly, the ability of drugs to lower ICSS thresholds has considerable predictive validity for evaluating their abuse potential (Negus and Miller, 2014). High doses of nicotine inhibit the function of brain reinforcement systems and elevate ICSS thresholds, which may reflect an anhedonic or aversive effect (Fowler et al., 2011; Kenny et al., 2003; Spiller et al., 2009). Nicotine's aversive effects may contribute to its abuse liability because they can limit the amount of nicotine that is consumed (for review, see Fowler and Kenny, 2013; Verendeev and Riley, 2013).

Our laboratory has used ICSS to evaluate the abuse-related effects of smokeless tobacco extracts, e-cigarette liquids, and isolated non-nicotine constituents (e.g., minor alkaloids) (Harris et al., 2018a; Harris et al., 2017; Harris et al., 2018b; Harris et al., 2012; Harris et al., 2015b; LeSage et al., 2016). Despite the sensitivity and utility of ICSS in studying addictive drugs in general and non-nicotine constituents in particular, effects of harmaline, norharmaline, and harmaline have not been evaluated in this model. To this end, the primary goal of this study was to evaluate the acute effects of these β -carbolines on ICSS in rats. A secondary goal was to evaluate the ability of norharmaline to influence nicotine's acute effects on ICSS.

2. Methods

2.1. Animals

Experimentally-naïve male Holtzman Sprague Dawley rats (Experiment 1) or age-matched male and female Sprague Dawley rats (Experiment 2) were obtained from Harlan/Envigo (Indianapolis, IN). Rats weighed 250–299 g (males) or 175–224 g (females) upon arrival. Rats were housed individually under a reversed 12-hr light/dark cycle and allowed unlimited access to water. Beginning one week after arrival, rats were food-restricted to ≈ 18 g/day

(males) or ≈ 16 g/day (females) rat chow to facilitate operant performance and avoid detrimental effects of long-term ad libitum feeding on health. All testing occurred during the dark (active) phase. Protocols were approved by the Institutional Animal Care and Use Committee of the Hennepin Healthcare Research Institute in accordance with the 2011 NIH Guide for the Care and Use of Laboratory Animals and the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Research Council 2003).

2.2. Drugs

(–)-Nicotine and harmane were obtained from Sigma (St Louis, MO). Norharmine hydrochloride and harmine hydrochloride were obtained from Santa Cruz Biotechnology (Dallas, TX). Nicotine was prepared in sterile saline and adjusted to a pH of 7.4 using dilute NaOH. Harmane, norharmine, and harmine were dissolved in dimethyl sulfoxide (DMSO) and saline. Nicotine and harmane doses are expressed as the base. Norharmine and harmine doses are expressed as the salt. All drugs were administered s.c. in a volume of 1 ml/kg.

2.3. Intracranial self-stimulation

Surgery, apparatus, and training procedure used here are described in detail elsewhere (Harris et al., 2013; Harris et al., 2010; Harris et al., 2011; Manbeck et al., 2013). Briefly, animals were anesthetized with i.m. ketamine (75 mg/kg)/ dexmedetomidine (0.025 mg) and implanted with an electrode in the medial forebrain bundle at the level of the lateral hypothalamus. Rats were later trained to respond for electrical brain stimulation using a modified version of the Kornetsky and Esposito (1979) discrete-trial current-threshold procedure (Markou and Koob, 1992). Each session lasted approximately 45 minutes and provided two dependent variables: ICSS thresholds (a measure of brain reinforcement function) and response latencies (a measure of non-specific (*e.g.*, motoric) effects).

2.4. Experiment 1: Effects of harmane on ICSS

Male rats ($N = 18$) were tested in daily ICSS sessions conducted Mon-Fri until thresholds were stable (less than 10% coefficient of variation over a 5-day period and no apparent trend). To habituate animals to the injection procedure, saline was administered 10 minutes prior to ICSS testing twice per week (Tuesdays and Fridays) for at least 1 session and until thresholds were stable. Rats were then injected with saline, 75% DMSO, or harmane at doses of 1.0, 2.5, 10.0, 20.0, and 30 mg/kg in 75% DMSO 10 minutes prior to each test session. These harmane doses were chosen based on their ability to produce addiction-related neurobiological effects (*e.g.*, increases in dopamine levels in the nucleus accumbens) (Arib et al., 2010; Baum et al., 1996) and to produce behavioral (*e.g.*, anxiolytic) effects in other models (Aricioglu and Altunbas, 2003; Aricioglu-Kartal et al., 2003; Celikyurt et al., 2013; Farzin et al., 2011). This pretreatment interval allowed for completion of the ≈ 45 min ICSS test while harmane levels were relatively high (half-life of harmane in rats is ≈ 30 min (Guan et al., 2001)). Injections were typically administered on Tuesday s and Fridays, provided that ICSS thresholds were within baseline range on intervening days, and doses were administered in a counterbalanced order.

2.5. Experiment 2: Effects of norharmane and harmine alone and nicotine + norharmane cocktails on ICSS.

2.5.1. Experiment 2a: Effects of norharmane and harmine alone on ICSS—

Male and female rats ($N = 17$, 8 males, 9 females) were prepared and trained for ICSS until thresholds were stable and habituated to s.c. saline injections as described in Experiment 1. Dose-response testing was subsequently conducted as described in Experiment 1, except that rats received either norharmane (half of the rats, split evenly between sexes) or harmine (the other half). During each dose-response determination, rats were injected with saline, 75% DMSO, or either norharmane or harmine at doses of 0.5, 1.0, 2.5, 5.0, and 10.0 mg/kg in 75% DMSO. As for the harmine doses used in Experiment 1, these norharmane and harmine doses were chosen based on their ability to activate the mesolimbic dopamine system or to produce behavioral effects (Aricioglu-Kartal et al., 2003; Baum et al., 1995; Farzin et al., 2011; Iurlo et al., 2001; MacInnes and Handley, 2002). Given that the half-lives of harmine and norharmane are ≈ 20 –45 min in rats (Fekkes et al., 2004; Guan et al., 2001; Zetler et al., 1974), use of a 10-minute pretreatment interval allowed for completion of behavioral testing while levels of these β -carbolines were still relatively high. Following completion of dose-response testing, rats were tested for ICSS under drug-free conditions for at least two weeks and until ICSS thresholds were stable. Rats then underwent the same procedure except that the β -carboline (norharmane versus harmine) was crossed-over within each subject.

2.5.2. Experiment 2b: Effects of nicotine + norharmane cocktails on ICSS—

Following completion of Experiment 2a, rats were tested under drug-free conditions for at least 2 weeks and until ICSS thresholds were stable. Dose-response testing was then conducted using the same within-subject crossover design described in Experiment 2a, except that rats received either nicotine alone (half the rats, split evenly between sexes), or nicotine + norharmane cocktails (the other half). For the nicotine alone condition, rats received saline, 10% DMSO, or nicotine at doses of 0.03, 0.125, 0.25, or 1.0 mg in 10% DMSO. For the nicotine + norharmane condition, rats received saline, 10% DMSO, 2.5 mg/kg norharmane in 10% DMSO, or cocktails of each of the above nicotine doses combined with 2.5 mg/kg norharmane in 10% DMSO. A 10% DMSO concentration was used because it was sufficient for dissolving this dose of norharmane, and because pilot studies indicated that the 75% DMSO concentration used in Experiments 1 and 2a attenuated the ICSS threshold-elevating effects of a 1.0 mg/kg dose of nicotine (data not shown). These nicotine doses produce either reductions (0.125, 0.25 mg/kg) or elevations (1.0 mg/kg) in ICSS thresholds when administered alone (e.g., Harris et al., 2015a; Harris et al., 2015b). This norharmane dose was used because it affects mesolimbic dopamine release and produces behavioral effects in other assays (Baum et al., 1995; Farzin et al., 2011), and because it did not increase ICSS thresholds (i.e., produce aversive/anhedonic effects) in Experiment 2a.

2.6. Statistics

Intracranial self-stimulation thresholds (in μ A) and response latencies (in seconds) were expressed as a percentage of baseline (i.e., mean during last 5 sessions prior to each dose-response determination). In all studies, paired-samples t-tests comparing the saline alone and

DMSO alone conditions showed no significant difference. These conditions were therefore combined to serve as a single vehicle control condition in all subsequent statistical analyses. In Experiments 1, ICSS threshold and latency data during harmaline dose-response testing were analyzed using separate one-factor, repeated measures ANOVA, followed by a Dunnett post hoc test comparing each harmaline dose to vehicle. We report the q ratio for each comparison in the Dunnett test, which is the difference between the two means divided by the standard error of that difference (computed from all conditions). Degrees of freedom for the ANOVAs were adjusted using the method of Geisser and Greenhouse to correct for any violations of sphericity. ICSS data during norharmaline and harmaline dose-response testing in Experiment 2a were analyzed in the same manner, except that sex was included as a between-subject factor in the ANOVAs. In Experiment 2b, data were analyzed using two-factor ANOVA with formulation (nicotine alone or nicotine + norharmaline) and nicotine dose as within-subject factors, followed by paired sample t -tests with a Bonferroni correction at each nicotine dose. Sex was not included as a factor in Experiment 2b due to the low number of rats of each sex ($n = 3-4$) that completed this protocol. Data for each formulation were also compared to vehicle using Dunnett's post hoc test. In cases in which a rat failed to respond for any ICSS current intensity, we assigned ICSS threshold and latency values based on those obtained in the animal achieving the highest ICSS threshold in that experiment (see Harris et al., 2018a; Harris et al., 2015a; Markou and Koob, 1991; Swain et al., 2020). This approach was used for 1 male in Experiment 1 at a harmaline dose of 20 mg/kg, 10 rats in Experiment 2a at harmaline doses of either 5 mg/kg (1 male), 10 mg/kg (3 males, 5 females), or both 5 and 10 mg/kg (1 female), and 1 female in Experiment 2b at the nicotine 1.0 mg/kg + norharmaline 2.5 mg/kg condition.

3. Results

3.1. Experiment 1: Effects of harmaline on ICSS

Two rats were lost to attrition during harmaline dose-response testing due to loss of ICSS headcap or loss of stable ICSS thresholds. Mean baseline ICSS thresholds and response latencies $\pm$ SEM in the remaining 16 male rats were $108.4 \pm 8.0 \mu\text{A}$ and 2.6 ± 0.1 seconds, respectively. There was a significant effect of dose on ICSS thresholds ($F(3.5, 51.9)=14.6$, $p < 0.0001$), with ICSS thresholds significantly elevated compared to vehicle at all harmaline doses 5.0 mg/kg (Dunnett $q = 3.5 - 7.9$, $p < 0.05$ or 0.01 ; Fig 1A). There was also a significant effect of dose on ICSS latencies ($F(3.6, 54.8)=4.2$, $p < 0.01$), with ICSS latencies elevated compared to vehicle at 10 mg/kg harmaline ($q = 3.0$, $p < 0.05$; Fig 1B). Although latencies also appeared elevated at 20 mg/kg harmaline compared to vehicle (Fig 1B), this difference was not statistically significant ($q = 2.9$, $p = 0.051$). Latencies did not differ between vehicle and any other harmaline dose (Fig 1B).

3.2. Experiment 2: Effects of norharmaline and harmaline alone and nicotine + norharmaline cocktails on ICSS.

3.2.1. Experiment 2a: Effects of norharmaline and harmaline alone on ICSS—

Due to attrition caused by removal of ICSS headcap or loss of stable ICSS thresholds, 2 rats were excluded from norharmaline testing and 1 rat from harmaline testing. Mean baseline ICSS thresholds and response latencies in the remaining rats ($n = 15$ and 16 for norharmaline and

harmine testing, respectively) did not differ between males and females for either β -carboline (Table 1).

There was a significant effect of norharmane dose on ICSS thresholds ($F(3.5, 45.5) = 11.2, p < 0.0001$), but no effect of sex or dose x sex interaction. Analysis of data collapsed across sex indicated that ICSS thresholds were elevated compared to vehicle at only the 10 mg/kg norharmane dose (Fig 2A). There was no effect of dose, sex, or dose x sex interaction on ICSS latencies (see Fig 2B for latency data collapsed across sex).

There was a significant effect of harmine dose on ICSS thresholds ($F(2.6, 36.0) = 38.9, p < 0.0001$) but no effect of sex. There was a significant dose x sex interaction ($F(5.0, 70.0) = 5.4, p < 0.001$), reflecting a trend for the 10 mg/kg dose to produce greater ICSS threshold-elevating effects in females compared to males (mean % baseline $\pm$ SEM = $221.0 \pm 3.9\%$ versus $163.5 \pm 18.1\%$). However, ICSS thresholds did not differ significantly between sexes at this dose or any other dose of harmine. Analysis of data collapsed across sex indicated that ICSS thresholds were significantly elevated compared to vehicle at all doses of harmine except the lowest dose (0.5 mg/kg) ($q = 4.3 - 7.4, p < 0.01$; Fig 2C). There was also a significant effect of dose on ICSS latencies ($F(3.4, 47.9) = 6.5, p < 0.001$), but no effect of sex or sex x dose interaction. Analysis of data collapsed across sex indicated that ICSS latencies were elevated compared to vehicle at harmine doses of 5.0 and 10.0 mg/kg ($q = 3.7$ and 5.0 , respectively, $p < 0.01$; Fig 2D).

3.2.2. Experiment 2b: Effects of nicotine + norharmane cocktails on ICSS—

Data were only analyzed for rats that completed both the nicotine alone and nicotine + norharmane conditions ($N = 7$, 4 males, 3 females). There were no differences between the nicotine alone and nicotine + norharmane conditions in terms of baseline ICSS thresholds ($93.0 \pm 10.6 \mu A$ versus $88.7 \pm 9.3 \mu A$) or response latencies (2.3 ± 0.07 sec versus 2.3 ± 0.12 sec). There was a significant effect of nicotine dose on ICSS thresholds ($F(1.8, 11.0) = 29.1, p < 0.0001$), a marginally significant effect of formulation ($p = 0.08$), and no nicotine dose x formulation interaction. Formulations did not differ significantly at any nicotine dose (Fig 3A). For the nicotine alone condition, ICSS thresholds were reduced compared to vehicle at 0.125 mg/kg nicotine ($q = 3.7, p < 0.05$) and elevated at 1.0 mg/kg nicotine ($q = 4.3, p < 0.05$). In contrast, no nicotine dose differed significantly from vehicle for the nicotine + norharmane condition (Fig 3A). There was no effect of nicotine dose, formulation, or nicotine dose x formulation interaction on ICSS latencies (Fig 3B).

4. Discussion

This study is the first to evaluate the abuse potential of the β -carbolines and MAO inhibitors harmine, norharmane, and harmine in an ICSS model. The main finding is that none of these β -carbolines lowered ICSS thresholds, suggesting a lack of abuse liability. Rather, all three β -carbolines elevated ICSS thresholds, suggesting aversive/anhedonic effects. Harmine and harmine also increased response latencies, indicating disruption of general motor function, albeit with reduced potency compared to their ICSS threshold-elevating effects. Finally, norharmane modestly attenuated the effects of nicotine on ICSS thresholds.

The lack of ICSS threshold-lowering effects of harmane, norharmane, and harmine contrasts with findings that some clinical MAO inhibitors (e.g., selegiline) can have addiction-like effects in other assays (e.g., place conditioning) in rats or mice (Porsolt et al., 1984; Wu and Zhu, 1999), although other studies reported no abuse potential or even aversive effects of clinical MAO inhibitors as measured using i.v. self-administration in rhesus monkeys (Winger et al., 1994) or conditioned taste aversion in rats (Buresova and Bures, 1987). Our data also contrast with a report that norharmane was reinforcing in an i.v. self-administration model in rats (Arnold et al., 2014). These discrepancies could reflect a number of differences between studies including dependent measure, route, and contingency. In addition, clinical MAO inhibitors and the present β -carbolines can produce various pharmacological effects other than MAO inhibition (e.g., Baker et al., 1992; Husbands et al., 2001), and differences in these effects between compounds could also account for discrepancies between studies. Further evaluation of the present β -carbolines in other addiction models (e.g., place conditioning) is needed to better understand their abuse potential.

The three β -carbolines differed in their potency to elevate ICSS thresholds. Potency for harmine was greater than for norharmane (minimal effective dose of harmine and norharmane expressed as base / salt = 0.85 / 1.0 mg/kg versus 8.2 / 10.0 mg/kg, respectively). Harmane appeared to have intermediate potency (minimal effective dose = 5.0 / 6.0 mg/kg), although this study was conducted in a different rat strain than the norharmane/harmine study and only used males rather than both sexes. However, the strain used in the harmane study (Holtzman Sprague Dawley) was derived from that used in the norharmane / harmine study (Sprague Dawley), and sex did not impact effects of harmine or norharmane on ICSS. As such, differences in these variables across studies likely had little or no impact on our findings. The apparent relative potency for harmane, norharmane, and harmine in elevating ICSS thresholds parallels their relative potency for inhibiting the MAO-A isozyme (Kim et al., 1997; Miralles et al., 2005), suggesting a role for MAO-A in the differential effects of these β -carbolines on ICSS.

In addition to their MAO inhibitory properties, harmane, norharmane, and harmine produce other pharmacological effects including inhibition of acetylcholinesterase, agonism at monoamine (e.g., dopamine) receptors, inverse agonism at the benzodiazepine site of GABA_A receptors, and binding at imidazoline (I₁ and I₂) receptors (e.g., Glennon et al., 2000; Husbands et al., 2001; Miralles et al., 2005; Rommelspacher et al., 1981). The ICSS threshold-elevating effects of the present β -carbolines may therefore reflect one or more of these pharmacological effects rather than MAO inhibition. Inverse agonism at GABA_A receptors represents a particularly plausible alternate mechanism, as other GABA_A inverse agonists (e.g., the β -carboline FG 7142) produce anhedonic and aversive effects in rats including elevations in ICSS thresholds (Cooper, 1986; Di Scala and Sandner, 1989; Evans and Lowry, 2007; Mumford and Holtzman, 1991). An involvement of I₂ receptors in our findings is also possible, as the relative potency of the present β -carbolines in elevating ICSS thresholds corresponds to their relative binding affinity at I₂ receptors in rat brain (Husbands et al., 2001). Evaluating the effects of pharmacological manipulations in this model (e.g., testing whether pretreatment with the GABA_A antagonist flumazenil blocks the ICSS

threshold-elevating effects of these β -carbolines) would clarify the role of MAO versus other mechanisms in our findings.

The elevation of ICSS latencies by harmaline and harmine is consistent with previous findings that these β -carbolines can suppress motor function in rats as measured using openfield locomotor activity or inactive lever pressing in an operant procedure (Giacobbo et al., 2020; Goodwin et al., 2015; Qiu et al., 2015). The lower potency of harmaline and harmine to elevate ICSS latencies compared to ICSS thresholds indicates that motoric effects of these compounds did not confound their effects on thresholds, at least at lower doses. In addition, ICSS thresholds and response latencies were not reliably correlated at doses of harmaline or harmine that did elevate ICSS latencies (e.g., Pearson's $r = 0.18$, $p = 0.50$ for the 5.0 mg/kg dose of harmine in Experiment 2a), consistent with previous reports that ICSS thresholds are relatively insensitive to response rates in the present discrete-trial current-threshold procedure (Harris et al., 2010; Harrison and Markou, 2001; Markou and Koob, 1992). Motoric effects of harmaline and harmine therefore cannot account for their aversive/anhedonic effects in this study.

Our findings complement a report that injections of harmine (15 mg/kg/day) produced anhedonic effects in a sucrose preference test in rats (Giacobbo et al., 2020). In contrast, other studies found that repeated administration of the same harmine dose did not produce anhedonia in rats as measured by consumption of sweet food, and actually blocked the ability of chronic mild stress to elicit anhedonia in this model (Abelaira et al., 2013; Fortunato et al., 2010b). Harmine and harmaline also produced antidepressant effects in rats in the forced swim test (Aricioglu and Altunbas, 2003; Fortunato et al., 2009), and all three of the current β -carbolines attenuated anhedonia- or depression-like behavior in mice as measured using the forced swim test, the tail suspension test, and/or sucrose preference (Farzin and Mansouri, 2006; Liu et al., 2017). Effects of harmaline, norharmaline, and harmine on measures of anhedonia or depression may therefore depend on the dependent measure, dosing regimen, species, or other factors.

The modest attenuation of nicotine's effects on ICSS by norharmaline was unexpected based on prior findings indicating that norharmaline and other MAO inhibitors can potentiate nicotine's addiction-related effects. For example, norharmaline increased nicotine's reinforcing effects in an i.v. self-administration model in male Sprague Dawley rats (Arnold et al., 2014). However, our findings complement a report that norharmaline reduced cocaine self-administration in rats (Cappendijk et al., 2001), although it is unclear whether those findings reflected a reduction of cocaine reinforcement *per se* versus non-specific (e.g. motoric) effects of norharmaline. Further studies evaluating effects of a range of doses of norharmaline, harmaline, and/or harmine on nicotine's effects on ICSS are needed to confirm our initial finding and further characterize interactions between nicotine and these β -carbolines in this model.

The current findings have implications for the regulation of tobacco products by the FDA. To the extent that our findings are indicative of the abuse potential of norharmaline, harmaline, and harmine in humans, they suggest that FDA-mandated increases in levels of these β -carbolines may reduce the abuse liability of tobacco products by increasing their aversive

effects and/or inhibiting the abuse potential of nicotine. Further evaluation of the effects of these β -carbolines in this and other addiction models is needed to further evaluate regulatory implications of our findings.

Sex can influence the abuse potential of nicotine in humans and animals (Chaudhri et al., 2005; Grebenstein et al., 2013; Perkins, 2009), and sex differences in certain behavioral effects (e.g., locomotor activity) have been reported for clinical MAO inhibitors and other β -carbolines in rats (Biegon et al., 1979; Meng and Drugan, 1993). We therefore included both sexes in Experiment 2 in order to model the heterogeneity of smokers and to allow for an *exploratory* evaluation of sex differences in the acute effects of norharmane and harmine on ICSS (i.e. our study was not powered specifically to detect sex differences). We found no significant sex differences in the effects of either β -carboline on ICSS thresholds or latencies. However, there was a non-significant trend for the highest dose of harmine to have greater ICSS threshold-elevating effects in females than in males. Data from the present study will be useful to power future studies to specifically evaluate potential sex differences in this model.

In conclusion, our findings suggest that the β -carbolines norharmane, harmine, and harmine produced only aversive/anhedonic effects in an ICSS model. Future work evaluating the addiction-related effects of these and other MAO inhibitors present in smoke (e.g., 2-naphthylamine), particularly when administered at lower, clinically relevant concentrations and in combination with nicotine as occurs during tobacco use, is needed to further understand the role of MAO inhibition in tobacco dependence. In addition, our approach for evaluating nicotine + norharmane mixtures in the current study involved adjusting the nicotine dose while keeping the norharmane dose constant, which is somewhat analogous to comparison of cigarettes with different levels of nicotine but the same level of norharmane. It would also be of interest to administer mixtures of nicotine and MAO inhibitors in fixed proportions, so that doses of both components increase or decrease together. This alternate approach would model changes in nicotine and MAO inhibitor exposure that occur due to changes in smoking topography of a cigarette, which causes levels of all constituents to change in parallel. This approach would also facilitate the use of certain analyses, such as isobologram analyses (e.g., Huang et al., 2019), to evaluate interactions between constituents. Together, such future work would also indicate whether FDA product standards for β -carbolines or other MAO inhibitors may be useful for moderating the abuse liability of tobacco products.

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Highlights

1. We tested effects of β -carboline MAO inhibitors found in cigarette smoke on ICSS.
2. Neither harmine, norharmine, or harmine had abuse liability in this assay.
3. All three β -carbolines had aversive/anhedonic effects.
4. Norharmine attenuated nicotine's acute effects on ICSS.

Figure 1.

ICSS thresholds (A) and response latencies (B) (expressed as percent of baseline, mean $\pm$ SEM) following injection of harmaline (N = 16, all male).*** Different from 0 mg/kg at that dose, $p < 0.05$ or 0.01.

Figure 2.

ICSS thresholds and response latencies (expressed as percent of baseline, mean $\pm$ SEM) following injection of norharmine (A and B) (N = 15, 8 males, 7 females) or harmine (C and D) (N = 16, 8 males, 8 females). Data shown are collapsed across sex. *** Different from 0 mg/kg, $p < 0.05$ or 0.01.

Figure 3.

ICSS thresholds (A) and response latencies (B) (expressed as percent of baseline, mean $\pm$ SEM) following injection of cocktails of nicotine (0–1.0 mg/kg) + norharmane (0 or 2.5 mg/kg) (N = 7, 4 males, 3 females). Data shown are collapsed across sex. * Significantly different from 0 mg/kg nicotine for that formulation, $p < 0.05$.

Table 1.

Mean ($\pm$ SEM) baseline ICSS thresholds (in μ A) and response latencies (in sec) in males and females during norharmane and harmine testing in Experiment 2a.

	Male		Female	
	Threshold (μ A)	Latency (sec)	Threshold (μ A)	Latency (sec)
Norharmane	89.1 $\pm$ 14.7	2.6 $\pm$ 0.1	89.3 $\pm$ 11.1	2.6 $\pm$ 0.1
Harmine	86.6 $\pm$ 10.2	2.5 $\pm$ 0.2	82.1 $\pm$ 11.5	2.4 $\pm$ 0.2