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Prior nicotine self-administration attenuates subsequent dopaminergic deficits of methamphetamine in rats: Role of nicotinic acetylcholine receptors

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Abstract

Preclinical studies have demonstrated that oral nicotine exposure attenuates long-term dopaminergic damage induced by toxins, including repeated, high doses of methamphetamine. It is suggested that alterations in nicotinic acetylcholine receptor (nAChR) expression, including $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ subtypes, likely contribute to this protection. The current study extended these findings by investigating whether nicotine self-administration in male, Sprague-Dawley rats 1) attenuates short-term dopaminergic damage induced by methamphetamine and 2) causes alterations in levels of $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ nAChR subtypes. The findings indicate that nicotine self-administration (0.032 mg/kg/infusion for 14 days) *per se* did not alter $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ nAChR expression or dopamine transporter (DAT) expression and function. Interestingly, prior nicotine self-administration attenuated methamphetamine-induced decreases in DAT function when assessed 24 h, but not 1 h, after methamphetamine treatment (4×7.5 mg/kg/injection). The ability of nicotine to attenuate the effects of methamphetamine on DAT function corresponded with increases in $\alpha 4\beta 2^*$, but not $\alpha 6\beta 2^*$, nAChR binding density. Understanding the role of nAChRs in methamphetamine-induced damage has the potential to elucidate mechanisms underlying the etiology of disorders involving dopaminergic dysfunction, as well as to highlight potential new therapeutic strategies for prevention or reduction of dopaminergic neurodegeneration.

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Keywords

Nicotine self-administration; methamphetamine; nicotinic acetylcholine receptors; dopamine transporter; rat

Introduction

Neurodegenerative diseases result in the loss of nerve structure and function; for instance, several conditions result in dopaminergic neurodegeneration, including Parkinson's disease and the use and abuse of some drugs like methamphetamine. Although these diseases manifest with different clinical features, many of the disease processes at the cellular level appear to be similar (McCann et al., 1998; Sekine et al., 2001; Volkow et al., 2001; Volz et al., 2007; Vaughan and Foster, 2013; Zuo and Motherwell, 2013; Kousik et al., 2014). For instance, the primary pathology of Parkinson's disease is well documented and includes the loss of dopaminergic nerve terminal markers in the caudate/putamen (for review, see Granado et al., 2013). A similar phenomenon has been observed in several preclinical studies demonstrating that methamphetamine-induced deficits in striatal dopaminergic markers [e.g., levels of tyrosine hydroxylase, dopamine transporter (DAT) and dopamine] are likely related to degeneration of dopaminergic axon terminals (Seiden et al., 1976; Hotchkiss and Gibb, 1980; Wagner et al., 1980; Brunswick et al., 1992; Kokoshka et al., 1998; Ares-Santos et al., 2014).

Given the similarities in dopaminergic damage between Parkinson's disease and methamphetamine use, it is not surprising that recent epidemiological work demonstrates that amphetamine/methamphetamine abusers have an increased risk of developing Parkinson's disease (Callaghan et al., 2010, 2012; Curtin et al., 2015). Interestingly, a growing body of evidence suggests that nicotine might be a useful therapeutic treatment for prevention or reduction of dopaminergic neuronal neurodegeneration (for review, see Quik et al., 2012). For instance, clinical findings indicate that there is a decreased incidence of Parkinson's disease among cigarette smokers relative to non-smokers (Chen et al., 2010). In addition, preclinical findings in both rodent and nonhuman primate models demonstrate that nicotine treatment attenuates nigrostriatal damage induced by toxins, including repeated, high-doses of methamphetamine (Ryan et al., 2001; O'Neill et al., 2002; Quik et al., 2007; Picciotto and Zoli, 2008; Vieira-Brock et al., 2015).

Several studies have investigated mechanisms underlying the neuroprotective effect of nicotine (Maggio et al., 1998; Roceri et al., 2001; Newman et al., 2002). One mechanism that has been the focus of considerable research is that nicotine has been shown to modulate dopaminergic neurotransmission mainly by enhancing dopamine release, through acting as an agonist at nicotinic acetylcholine receptors (nAChRs) located on dopaminergic cell bodies and terminals in both the mesocorticolimbic and nigrostriatal systems (Clarke and Pert, 1985; Wonnacott et al., 2000; Gotti et al., 2010). nAChRs are composed of α ($\alpha 2$ - $\alpha 10$) and β ($\beta 2$ - $\beta 4$) subunits, which assemble into pentameric structures (Anand et al., 1991); however, only $\alpha 3$ - $\alpha 7$ and $\beta 2$ - $\beta 4$ are expressed on dopamine neurons (Klink et al., 2001; Azam et al., 2002; Grady et al., 2007).

The exact composition of nAChR subtypes mediating nicotine-evoked dopamine release is the subject of ongoing investigation, although the $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ nAChR subtypes are clearly involved (where * represents subunits other than those indicated that might be present in the heteropentameric receptors; (Salminen et al., 2004; Scholze et al., 2007). For instance, chronic nicotine administration alters cell surface expression and function (i.e. dopamine release) of the $\beta 2^*$ nAChR subtypes (Perez et al., 2013; Marks et al., 2014). Specifically, nicotine has been demonstrated to up-regulate $\alpha 4\beta 2^*$ and down-regulate $\alpha 6\beta 2^*$ nAChRs in many brain regions and across species (Perry et al., 1999; Perez et al., 2008; Govind et al., 2009; Quik et al., 2010; Marks et al., 2014). Thus, it is likely that nicotine-induced alterations to $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ nAChR subtypes, including both expression and the capacity of nicotine to evoke dopamine release, contributes to the ability of nicotine to attenuate nigrostriatal damage induced by toxins.

In support of this notion, studies examining the effects of chronic nicotine on dopaminergic deficits induced by neurotoxic agents, including methamphetamine, have suggested an important role for $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ nAChRs (Ryan et al., 2001; Huang et al., 2009; Quik et al., 2013; Vieira-Brock et al., 2015). The majority of these studies examined the ability of chronic nicotine to attenuate long-term (i.e. 7-day) toxin-induced dopaminergic deficits; the current study extends these findings by examining whether chronic nicotine attenuates short-term (i.e. 1- and 24-h) effects of methamphetamine, in order to elucidate the relationship between early and long-term alterations in nicotinic and dopaminergic markers. In addition, most studies administer chronic nicotine intravenously (i.v.) in a non-contingent manner or via drinking water, to assess changes in levels of $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ nAChRs (Perez et al., 2008; Huang et al., 2009; Marks et al., 2014); it is unclear whether nicotine administered contingently (i.e. self-administration) alters $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ nAChR expression. Thus, the goals of the current study were to examine whether: 1) nicotine self-administration alters expression of $\alpha 4\beta 2^*$ and/or $\alpha 6\beta 2^*$ nAChRs; and 2) prior nicotine self-administration attenuates acute (i.e. 1- and 24-h) methamphetamine-induced dopaminergic deficits (i.e. dopamine transporter (DAT) function and expression).

Methods

Subjects

A total of 64 Male Sprague-Dawley rats (275-300 g; Charles River Breeding Laboratories) were housed individually per cage and maintained in a temperature and humidity controlled environment on a 14:10 h light/dark cycle with free access to water. Twenty-four hours prior to the initiation of operant training, all rats were food-restricted to 90% of their free-feeding body weight for the duration of the experiments (with the exception of recovery time after surgery and after the 14-day self-administration period). Approximately 1 hour after the end of the last self-administration session (i.e. day 14), one group of rats were sacrificed and tissue was collected (see below). The other two groups of rats received either methamphetamine or saline ~1 h after the end of the last self-administration session and were sacrificed either 1 or 24 h after treatment, depending on the group. For these experiments, rats were individually housed in plastic cages and were maintained in a warm ambient temperature to enhance methamphetamine-induced hyperthermia. Rats received

methamphetamine (4×7.5 mg/kg/injection, s.c.; 2-h intervals) or saline (4×1 ml/kg/injection, s.c.; 2-h intervals). Core (rectal) body temperatures were recorded using a digital rectal thermometer (Physitemp Instruments, Clifton, NJ). Rectal temperatures were recorded immediately before the first injection and then 30 min before and after every injection of either methamphetamine or saline. All experiments were approved by the University of Utah Institutional Animal Care and Use Committee, in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

Apparatus

All experimental sessions were conducted in an operant chamber ($30.5 \times 25.5 \times 30.5$ cm; Coulbourn Instruments, Allentown, PA) located within a sound-attenuating cubicle ($79 \times 53 \times 53$ cm; Coulbourn Instruments). Each chamber was equipped with a food pellet hopper, two retractable levers, and a house light. An infusion pump connected to a liquid swivel suspended outside of the operant chamber delivered drug or saline through a polyethylene tube located within a spring leash tethered to a rat.

Food Training

Before surgery, rats were trained to lever press for food in daily 14-h sessions. First, rats were trained under a fixed ratio (FR) 1 schedule of reinforcement whereby pressing on either lever resulted in the delivery of a food pellet. When at least 100 pellets were received in a session, only responding on the active lever (for some rats the active lever was the left lever and for others, the right lever) was reinforced. Responses on the inactive lever were counted but had no programmed consequence. Once at least 100 pellets were received in a session, the FR ratio was increased as follows: FR2, FR3, and the terminal condition of FR5. Food training was completed upon stable responding for 2 consecutive sessions, while responding under the FR 5 schedule (this training required ~6-9 sessions).

Surgery

Rats were surgically implanted with a chronic indwelling jugular catheter (constructed in the laboratory as described previously: Frankel et al., 2011) in the right jugular vein under ketamine:xyalazine (90:7 mg/kg; i.p.) anesthesia. The outlet of the catheter was implanted s.c. in the back, and the free end of the Silastic tubing was inserted ~25 mm into the right jugular vein and secured to the surrounding tissue with sutures. Each rat received flunixin meglumine (s.c.) on the day of the surgery. Immediately after surgery and daily thereafter, catheters were infused with 0.05 ml of heparinized saline prior to the start of each session and with 0.1 ml of the antibiotic cefazolin followed by 0.05 ml of heparinized saline and heparinized glycerol after the completion of each session. If at any point during the experiment catheter leaks or abnormal shifts in self-administration behavior were observed, rats received xylazine through the catheter. Rats with patent catheters exhibited clear loss of muscle tone within a few seconds of the i.v. injection. Data collected from animals with nonpatent catheters were excluded from the data analyses. All rats were allowed to recover for a minimum of 3 days after surgery before the start of self-administration sessions.

Drug Self-Administration

All rats were tested in daily 23-h sessions during which responses (FR5) on the active lever resulted in the delivery of nicotine (0.032 mg/kg/infusion, (O'Dell et al., 2007) or saline (infusion duration 5-6 s, corresponding to 0.01-0.02 ml). Each infusion was followed by a 20-s timeout during which both levers were retracted. Rats self-administered either nicotine or saline for 14 consecutive sessions, a sufficient period to allow for stabilization of responding, defined as three consecutive sessions whereby the number of infusions did not change by more than $\pm 20\%$ for the mean number of infusions for an individual rat and no increasing or decreasing trend in overall group mean responding.

Tissue Preparation

Rats were sacrificed either ~1 h, 9 h (i.e. 1 h after the last methamphetamine or saline treatment), or 33 h (i.e. 24 h after the last methamphetamine or saline treatment) after the end of the last self-administration session. Brains were hemisected, and the right side rapidly removed and frozen in isopentane on dry ice and stored at -80°C . The left hemisphere was used for radioligand uptake experiments (see below). Frozen right hemisected brains were sliced at 12 μm thick at the level of the anterior striatum and nucleus accumbens (1.5 mm from bregma, Paxinos and Watson 6th edition) using a cryostat. Four slices (per rat) were mounted on Superfrost® Plus glass micro slides (VWR International, Radnor, PA) and stored at -80°C for subsequent use in autoradiography assays.

Synaptosomal [^3H]dopamine Uptake

Synaptosomal uptake of [^3H]dopamine through DAT was determined in all three groups and according to methods reported previously (Fleckenstein et al., 1997; Hadlock et al., 2009). In brief, freshly dissected striatal tissue was homogenized in ice-cold 0.32 M sucrose and centrifuged (800g for 12 min; 4°C). The supernatant (S1) was then centrifuged (22,000g for 15 min; 4°C), and the resulting pellet (P2) was resuspended in ice-cold 0.32 M sucrose. Assays were conducted in modified Krebs' buffer (126 mM NaCl, 4.8 mM KCl, 1.3 mM CaCl_2 , 16 mM sodium phosphate, 1.4 mM MgSO_4 , 11 mM dextrose, 1 mM ascorbic acid; pH 7.4). Each assay tube contained synaptosomal tissue (i.e., resuspended P2 obtained from 1.5 mg of original wet weight striatal tissue) and 1 mM pargyline. Nonspecific values were determined in the presence of 1 mM cocaine. After preincubation of assay tubes for 10 min at 37°C , assays were initiated by the addition of [^3H]dopamine (0.5 nM final concentration). Samples were incubated at 37°C for 3 min, then filtered through Whatman GF/B filters soaked previously in 0.05% polyethylenimine. Filters were washed rapidly 3 times with 3 ml of ice-cold 0.32 M sucrose using a Brandel filtering manifold. Radioactivity trapped in filters was counted using a liquid scintillation counter. Remaining resuspended P2 samples were assayed for protein concentrations according to the previous methods (Lowry et al., 1951).

^{125}I -RTI-55 Autoradiography

DAT density was assessed via ^{125}I -RTI-55 binding to striatal core slices as previously described (O'Dell et al., 2012). Briefly, slides were thawed on a slide warmer (5-10 min) and pre-incubated in buffer-sucrose (10 mM sodium phosphate, 120 mM sodium chloride, 320

mM sucrose, pH 7.4) containing 100 nM fluoxetine at room temperature for 5 min. Slides were then incubated for 2 h in buffer-sucrose containing 25 pM ^{125}I -RTI-55 (2200 Ci/mmol, PerkinElmer, Watham, MA). In a previous study conducted in the same laboratory, nonspecific binding was demonstrated by slides incubated in buffer-sucrose containing 25 pM ^{125}I -RTI-55, 100 nM fluoxetine, and 100 μM nomifensine (Vieira-Brock et al., 2015). Slides were rinsed twice in ice-cold buffer and distilled water for 2 min and air-dried. Sample slides and standard ^{125}I microscale slides (American Radiolabeled Chemicals, St. Louis, MO) were exposed to Kodak MR film (Eastman Kodak Co., Rochester, NY, USA) for 24 hours.

^{125}I - $\alpha\text{ConotoxinMII}$ (αCtxMII) Autoradiography

$\alpha 6\beta 2^*$ nAChR density was assessed via ^{125}I - αCtxMII (2200 Ci/mmol) {Whiteaker, 2000 #568} binding to striatal core slices as previously described (Lai et al., 2005; Huang et al., 2009). Briefly, slides were thawed on a slide warmer (5-10 min) and pre-incubated in buffer A (pH 7.5, 20 mM HEPES, 144 mM NaCl, 1.5 mM KCl, 2 mM CaCl_2 , 1 mM MgSO_4 , 0.1% BSA, and 1 mM phenylmethylsulfonyl fluoride) at room temperature for 15 min. The buffer was removed and replaced with fresh buffer A for an additional 15 min. Slides were then incubated for 1 h in buffer B (20 mM HEPES, 144 mM NaCl, 1.5 mM KCl, 2 mM CaCl_2 , 1 mM MgSO_4 , 0.2% BSA, 5 mM EDTA, 5 mM EGTA, and 10 $\mu\text{g/ml}$ each of aprotinin, leupeptin, and pepstatin A) containing 0.5 nM ^{125}I - αCtxMII . In a previous study conducted in the same laboratory, nonspecific binding was demonstrated by slides incubated in 0.5 nM ^{125}I - αCtxMII buffer B also containing 0.1 mM nicotine (Sigma-Aldrich, St. Louis, MO). Slides were rinsed in room temperature buffer A for 10 min, then in ice-cold buffer A for another 10 min, followed by 2×10 min in $0.1 \times$ ice-cold buffer A, and finally in 4°C distilled water for 2×10 s and air-dried. Sample slides and standard ^{125}I microscale slides (American Radiolabeled Chemicals, St. Louis, MO) were exposed to Kodak MR film (Eastman Kodak Co., Rochester, NY, USA) for 4 days.

^{125}I -epibatidine Autoradiography

$\alpha 4\beta 2^*$ nAChR density was assessed via ^{125}I -epibatidine binding to striatal core slices as previously described (Lai et al., 2005; Huang et al., 2009). Briefly, slides were thawed on a slide warmer (5-10 min) and pre-incubated in binding buffer (50 mM Tris, 120 mM NaCl, 5 mM KCl, 2.5 mM CaCl_2 , 1.0 mM MgCl_2 , pH 7.5) plus 100 nM αCtxMII {Cartier, 1996 #569} (used to inhibit epibatidine binding to $\alpha 6\beta 2^*$ nAChR) at room temperature for 30 min. Slides were then incubated for 40 min in binding buffer containing 0.015 nM ^{125}I -epibatidine (2200 Ci/mmol, PerkinElmer, Watham, MA) in the presence of 100 nM αCtxMII . In a previous study conducted in the same laboratory, nonspecific binding was determined by slides incubated in binding buffer containing 0.015 nM ^{125}I -epibatidine plus 0.1 mM nicotine (Vieira-Brock et al., 2015). Slides were rinsed twice in ice-cold buffer for 5 min followed by a 10 s rinse in distilled water and air-dried. Sample slides and standard ^{125}I microscale slides (American Radiolabeled Chemicals, St. Louis, MO) were exposed to Kodak MR film (Eastman Kodak Co., Rochester, NY, USA) for 24 h.

Drugs

(±)-Methamphetamine hydrochloride (dose described as the free-base form; Research Triangle Institute, Research Triangle Park, NC) and (–)-nicotine hydrogen tartrate salt (adjusted to pH 7.2–7.4; Sigma, St Louis, MO) were dissolved in 0.9% sterile saline. Ketamine (Hospira Inc., Lake Forest, IL) and xylazine (Sigma-Aldrich, St. Louis, MO) were used to anesthetize animals. The antibiotic cefazolin (10 mg/ml; Schein Pharmaceutical, Florham Park, NJ) was dissolved in heparinized saline (63.33 U/ml; Sigma-Aldrich). Flunixin meglumine (1.1 mg/kg; MWI Veterinary Supply, Meridian, ID) was used for postsurgical analgesia.

Data Analyses

Self-administration data are expressed as the average ($\pm$ S.E.M.) infusions earned and plotted as a function of session. Synaptosomal [3H] dopamine uptake data are expressed as average ($\pm$ S.E.M.) fmol of radioligand per microgram (μ g) protein and plotted as a function of treatment group. Comparisons were made within an experiment (versus across separate experiments), such that animals within a given experiment were treated concurrently, tissues were processed simultaneously and assay conditions to which samples were exposed were identical. Optical densities from 4 replicate slices per rat were quantified using ImageJ software (National Institutes of Health, USA). Specific binding was obtained by subtracting mean density values from film background and converted to fmol/mg using the standard curve generated from 125 I standards. Statistical analyses were conducted with an ANOVA with post hoc Bonferroni's for multiple comparisons. For all tests, significance was set at $P = 0.05$.

Results

Responding (i.e. lever pressing) maintained by nicotine (0.032 mg/kg/infusion) was greater than for saline (Fig. 1A, 2A and 2C), and the average number of nicotine infusions and intake were similar across experiments. For example, the average number of nicotine infusions ($\pm$ SEM) earned during the final 3 self-administration sessions (i.e. sessions 12–14 for Figs. 1A, 2A, and 2C) were 21 ($\pm$ 4.7), 18 ($\pm$ 4), and 17 ($\pm$ 1.5), and the average nicotine intake (mg/kg) during the final 3 self-administration sessions (Figs. 1A, 2A, and 2C) was 0.7 ($\pm$ 0.2), 0.6 ($\pm$.1), and 0.6 ($\pm$.05).

Nicotine self-administration *per se* did not alter striatal DAT uptake as assessed ~1 h after the final self-administration session (Fig. 1B). In experiments where methamphetamine (4×7.5 mg/kg; s.c.) or saline ($4 \times$ ml/kg; s.c.) were administered ~1 h after the final self-administration session, prior nicotine did not attenuate methamphetamine-induced decreases in striatal DAT uptake as assessed 1 h after the last methamphetamine treatment (Fig. 2B). In contrast, when striatal DAT uptake was assessed 24 h after the last methamphetamine treatment, prior nicotine attenuated methamphetamine-induced decreases in striatal DAT uptake (Fig. 2D). Methamphetamine increased core body temperature and body temperatures were not different between methamphetamine-treated groups with a history of nicotine or saline self-administration (data not shown). For example, average body temperatures ($\pm$ SEM) across time after the first injection (saline or methamphetamine) were

as follows for rats sacrificed 1 h after the last treatment: saline/saline 37.9 ($\pm$ 12), saline/methamphetamine 39.0 ($\pm$ 30), nicotine/methamphetamine 38.9 ($\pm$ 14). Average body temperatures ($\pm$ SEM) across time after the first injection (saline or methamphetamine) were as follows for rats sacrificed 24 h after the last treatment: saline/saline 37.9 ($\pm$ 08), saline/methamphetamine 38.9 ($\pm$ 17), nicotine/methamphetamine 39.1 ($\pm$ 12).

The results presented in Table 1 indicate that nicotine alone did not alter striatal ^{125}I -epibatidine ($\alpha 4\beta 2^*$), ^{125}I - αCtxMII ($\alpha 6\beta 2^*$), or ^{125}I -RTI-55 (DAT) binding. Furthermore, under the conditions in the current study, methamphetamine alone did not alter binding densities of $\alpha 4\beta 2^*$ receptors, $\alpha 6\beta 2^*$ receptors, or DAT, as assessed either 1 or 24 h after the final methamphetamine treatment. However, in rats with prior nicotine self-administration and assessed 24 h after methamphetamine treatment, the binding density of $\alpha 4\beta 2^*$ receptors, but not $\alpha 6\beta 2^*$ receptors or DAT, was increased.

Discussion

The first major finding of the current study is that nicotine self-administration for 14 days (0.032 mg/kg/infusion) did not alter binding densities of $\alpha 4\beta 2^*$ receptors, $\alpha 6\beta 2^*$ receptors, or DAT expression and function. These findings are in contrast to other reports demonstrating that chronic nicotine up-regulates $\alpha 4\beta 2^*$ receptors and down-regulates $\alpha 6\beta 2^*$ receptors (Perez et al., 2008; Govind et al., 2009; Marks et al., 2014). This may be due to the route of administration (oral vs. i.v.), the duration of exposure, or total nicotine intake. With regard to the latter, in the current study the average nicotine intake (mg/kg) during the final 3 self-administration sessions ranged from 0.6 to 0.7 mg/kg/day. For comparison (albeit differences in routes of administration), human nicotine consumption reportedly varies widely from 10.5 – 78.6 mg/day, and thus 0.15 – 1.1 mg/kg/day (assuming an average 70 kg human; Benowitz and Jacob, 1984). However, in other studies, the doses of nicotine administered to induce changes in $\alpha 4\beta 2^*$ and $\alpha 6\beta 2^*$ receptor level were significantly larger (Perry et al., 1999; Perez et al., 2008; Huang et al., 2009). For example, one study administered nicotine for 14 days via osmotic minipumps that equated to a dose of ~ 6 mg/kg/day (Perry et al., 2007). In regard to DAT binding, the current findings are in concordance with another study reporting that chronic nicotine administered *in vivo* did not alter DAT expression and function in striatum (Collins et al., 2004; Marks et al., 2014). The current findings also indicate that under the current conditions (i.e., SA for 14 days beginning at PND63), expression of $\alpha 4\beta 2^*$ receptors, $\alpha 6\beta 2^*$ receptors, or DAT expression and function does not need to be altered at the time of methamphetamine treatment for nicotine to exert a protective effect as assessed 24 h later.

The second major finding of the current study is that prior nicotine self-administration attenuated the subsequent deficits in DAT function induced by methamphetamine when assessed 24 h, but not 1 h, after the last methamphetamine treatment. These findings are similar to those reported in a recent study that demonstrated that prior treatment with methamphetamine attenuated subsequent methamphetamine-induced decreases in DAT function assessed 24 h, but not 1 h, after treatment (McFadden et al., 2015). Taken together, these data suggest that the capacity of agents, including nicotine, to attenuate methamphetamine-induced alterations in DAT function might be dependent on how

methamphetamine itself is modulating the transporter. In other words, the effects of methamphetamine on dopaminergic parameters can be dependent on time evaluated after treatment. For example, repeated, high-dose methamphetamine treatment does not alter DAT immunoreactivity as assessed 1 h after treatment (Kokoshka et al., 1998); however, beginning ~ 6-12 h after treatment, high-molecular weight DAT complexes begin to form (Baucum et al., 2004; Hadlock et al., 2009, 2010), suggesting a time-response function of methamphetamine on DAT. Lastly, the current findings add to the growing body of literature suggesting that chronic nicotine treatment (i.e. administered across days) attenuates subsequent methamphetamine-induced dopaminergic deficits (Vieira-Brock et al., 2015). Although it has also been demonstrated that multiple, acute injections (i.e. administered across several hours) of nicotine attenuate methamphetamine-induced damage to dopamine terminals (Ryan et al., 2001), it is unclear if a single, acute injection of nicotine would similarly impact the effects of methamphetamine on dopaminergic deficits.

In the current study, methamphetamine-induced decreases in DAT function did not correspond to decreases in DAT expression, as assessed via ^{125}I -RTI-55 binding either 1 or 24 h after treatment. This finding is similar to another study (Kokoshka et al., 1998) that demonstrated that DAT uptake and binding do not always strictly correlate (i.e. the magnitude of the effect of methamphetamine on DAT function was greater than the effect on DAT expression). Furthermore, it is well established that methamphetamine-induced hyperthermia contributes to the effects on DAT and prevention of hyperthermia attenuates changes to DAT immunoreactivity (Metzger et al., 2000; Baucum et al., 2004). Thus, although methamphetamine increased core body temperature to a similar magnitude, regardless of history (i.e. nicotine or saline), it is possible that in the current study, the degree of methamphetamine-induced hyperthermia was not sufficient to impact DAT expression levels.

The third major finding of the current study is that the neuroprotective effect of nicotine at 24 h corresponded with increases in $\alpha 4\beta 2^*$, but no changes in $\alpha 6\beta 2^*$, expression level (i.e. $\alpha 6\beta 2^*$ receptor binding density was similar regardless of drug treatment), although it is likely that additional mechanisms other than alterations to $\alpha 4\beta 2^*$ receptor expression play a role in the neuroprotective effects of nicotine [i.e. changes to nAChR function and/or other nAChR subtypes that regulate dopamine release (Kaiser and Wonnacott, 2000; Buisson and Bertrand, 2002; Bordia et al., 2015)]. One mechanism whereby nicotine might attenuate methamphetamine-induced neurotoxicity is related to how nicotine stimulates dopamine release (Kulak et al., 1997; Kaiser et al., 1998; Salminen et al., 2004), alters dopamine concentrations, and thus formation of reactive oxygen species (an important component of neurotoxicity; Giovanni et al., 1995, Yamamoto and Zhu, 1998, Graham, 1978). Interestingly, $\alpha 4\beta 2^*$ nAChR activation increases tonic (versus phasic) dopamine release (Nashmi et al., 2007; Meyer et al., 2008; Perez et al., 2010) and increases in tonic dopamine levels can inhibit phasic, high amplitude dopamine release (Grace, 1991, 1995). To the extent that dopaminergic terminals that cause phasic dopamine release are more susceptible to the neurotoxic effects of methamphetamine (Howard et al., 2013), $\alpha 4\beta 2^*$ nAChR-mediated increases in tonic dopamine release might protect terminals from the effects of methamphetamine.

In conclusion, the current findings provide additional evidence to support preclinical observations that the neuroprotective effect of nicotine on methamphetamine-induced dopaminergic deficits extends to nicotine self-administration models. Given the large number of methamphetamine users worldwide (i.e. amphetamine-type stimulants are the second most widely used class of drugs worldwide; United Nations Office on Drugs and Crime, 2013), elucidating mechanisms involved in methamphetamine-induced damage might also shed light on the pathology of Parkinson's disease and highlight potential new therapeutic strategies, such as nicotinic agonists, for reduction of dopaminergic neurodegeneration.

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Fig. 1. Effects of prior nicotine (0.032 mg/kg/inf) or saline self-administration (A) on striatal DAT uptake (B). Each condition represents the mean $\pm$ SEM of 8-10 rats. Horizontal axes: Ticks indicate daily consecutive sessions or treatment groups. Vertical axes: mean infusions earned ($\pm$ SEM) or fmol of [3 H]dopamine per μ g protein.

Fig. 2.

Effects of prior nicotine (0.032 mg/kg/inf) or saline self-administration (A, C) on methamphetamine-induced decreases in striatal DAT uptake (B,D). Each condition represents the mean $\pm$ SEM of 6-11 rats. Horizontal axes: Ticks indicate daily consecutive sessions or treatment groups. Vertical axes: mean infusions earned ($\pm$ SEM) or fmol of [3 H]dopamine per μ g protein. * $p < 0.05$ relative to saline/saline.

Table 1

The effects of nicotine and methamphetamine on striatal autoradiography binding (fmol/mg) to $\alpha 4\beta 2^*$ nAChR (^{125}I -epibatidine), $\alpha 6\beta 2^*$ nAChR (^{125}I - αCtxMII), and DAT (^{125}I -RTI-55)

	^{125}I -epibatidine	^{125}I - αCtxMII	^{125}I -RTI-55
<u>Nicotine Self-administration</u>^c			
Saline	5.5 (± 0.26) ^a	2.3 (± 0.11)	5.6 (± 0.16)
Nicotine	5.7 (± 0.23)	2.4 (± 0.11)	5.6 (± 0.14)
<u>Nicotine Self-administration + Saline/METH (1 hour)</u>^d			
Saline/Saline	6.7 (± 0.22)	1.9 (± 0.12)	8.4 (± 0.34)
Saline/METH	6.9 (± 0.56)	1.9 (± 0.13)	9.3 (± 0.18)
Nicotine/METH	7.7 (± 0.12)	1.9 (± 0.04)	8.9 (± 0.17)
<u>Nicotine Self-administration + Saline/METH (24 hour)</u>^e			
Saline/Saline	8.2 (± 0.32)	2.3 (± 0.16)	10.7 (± 0.28)
Saline/METH	7.3 (± 0.54)	2.3 (± 0.11)	10.1 (± 0.33)
Nicotine/METH	8.7 (± 0.22)^b	2.2 (± 0.09)	9.5 (± 0.30)

^a Average density as expressed in fmol/mg ($\pm$ S.E.M.) of 6-11 rats per group

^b Values significantly different from Saline/METH values ($P < 0.05$)

^c Tissue collected ~1 hour after last nicotine self-administration session

^d Tissue collected ~9 hour after last nicotine self-administration session (1 hour after last saline or METH treatment)

^e Tissue collected ~33 hour after last nicotine self-administration session (24 hour after last saline or METH treatment)