Reinforcement enhancement by nicotine in adult rats: behavioral selectivity and relation to mode of delivery and blood nicotine levels

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Abstract

Rationale Reinforcement-enhancing effects of nicotine occur in human subjects and laboratory rats. However, the doses used in animal studies typically exceed smoking-associated levels of exposure, and generalized behavioral activation by nicotine can potentially confound data interpretation.

Methods During daily 60-min sessions, male adult rats pressed an “active” lever to illuminate a brief cue light. Pressing on either the active or inactive lever retracted both levers for 60 s. Nicotine (0.025–0.2 mg/kg) was given either by continuous intravenous (IV) infusion, or spaced IV pulses (3-s or 30-s/pulse), or pre-session subcutaneous (SC) injection.

Results Almost all rats responded preferentially for the cue light for several weeks. After several home-cage nicotine injections, reinforcement enhancement occurred even within the first nicotine test session. Nicotine increased active lever responding without altering inactive lever responding, with effects reliably observed at doses as low as 0.1 mg/kg SC or 0.1 mg/kg/session IV. Within the session, the 0.1 mg/kg dose maximally increased active lever responding by 2–3-fold, coinciding with serum levels of 25 ng/ml. Intravenous nicotine (tested at 0.1 mg/kg/60-min session) was equally effective whether delivered by continuous infusion or in a series of equally spaced 0.003 mg/kg pulses each of 3-s or 30-s duration.

Conclusions Low doses of nicotine can potentiate responding for a primary sensory reinforcer without producing a generalized increase in lever pressing. Reinforcer enhancement by

nicotine generalized to several modes of drug delivery, appeared to track circulating levels of drug, and occurred even at serum levels within the daytime range of moderate cigarette smokers.

Keywords Nicotine · Reinforcement enhancement · Route of administration · Pharmacokinetics · Tobacco · Smoking

Introduction

In the dual-reinforcement model proposed by Caggiula, Donny and co-workers, nicotine promotes tobacco smoking principally via (1) a primary positive reinforcing effect and (2) a reinforcement-enhancing effect, that is, by potentiating the reinforcing effects of smoking-associated cues (Caggiula et al. 2009; Donny et al. 2003). The positive reinforcing effects of nicotine are at best weak, once the drug is isolated from associated sensory cues—as shown both in animals (Caggiula et al. 2002; Chaudhri et al. 2005; Donny et al. 2003; Palmatier et al. 2006; Sorge et al. 2009) and in humans (Duke et al. 2015; Fulton and Barrett 2008; Jensen et al. 2016). In contrast, nicotine can strengthen non-drug reinforcers, both in animal and human subjects (Perkins et al. 2017; Rupprecht et al. 2015).

Tobacco smoking aside, reinforcement enhancement appears critical in maintaining intravenous self-administration (IVSA) of nicotine in animals (Rupprecht et al. 2015). In conventional IVSA procedures, each drug infusion is typically paired with a brief light cue appearing above the lever. Such visual stimuli can serve as weak unconditioned reinforcers (Caggiula et al. 2002; Sorge et al. 2009), and they can also become conditioned reinforcers by association with the drug (Palmatier et al. 2007a); both conditioned and unconditioned reinforcers can be strengthened by nicotine (Rupprecht et al. 2015).

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Reinforcement enhancement by nicotine was initially detected in an intracranial electrical self-stimulation procedure which was specifically designed to avoid the confounding effects of motor output (Clarke and Kumar 1984). However, many subsequent studies have used sensory stimuli (e.g., brief cue light illumination) which serve as either primary reinforcers or conditioned reinforcers; here, response rates on an “active” reinforcer-associated lever are compared with response rates on an “inactive” (control) lever. The rate of inactive lever pressing is intended to control for the potentially confounding effect of general behavioral activation, which is particularly evident after repeated nicotine exposure (Clarke 1987). Currently, the literature appears mixed with regard to behavioral specificity. Thus, nicotine has been reported to increase only active lever responding (Chaudhri et al. 2007; Liu et al. 2007; Olausson et al. 2004a, b; Weaver et al. 2012), or to selectively increase a related operant “observing response” (Raiff and Dallery 2006), but in several other studies, nicotine produced comparable or even larger proportional increases in inactive vs. active lever pressing (Barrett and Bevins 2013; Barrett and Odum 2011; Donny et al. 2003; Grimm et al. 2012; Palmatier et al. 2006; Swalve et al. 2015; Tronci et al. 2010).

Reports of nicotine-induced reinforcement enhancement in animals are based, seemingly without exception, on doses that would provide levels of drug exposure beyond the range typically experienced by tobacco smokers. This issue, documented below, is problematic since to our knowledge no published evidence exists to suggest that rat nicotinic cholinergic receptors are less sensitive than their human counterparts. A cigarette puff delivers approximately 0.001–0.003 mg/kg of nicotine, with lung-to-brain transit ensuing over many seconds or even minutes (Rose et al. 1999, 2010). In contrast, numerous animal studies have used rapid, high-dose bolus delivery (e.g., 0.03 mg/kg/infusion, given in 1 s) directly via the jugular vein (Caggiula et al. 2009; Chaudhri et al. 2007; Donny et al. 2003; Liu et al. 2007; Palmatier et al. 2007a), with transient concentrations of blood nicotine far exceeding the smoking range (Hieda et al. 1999).

Rapid nicotine spikes of this kind have been avoided in some studies through the use of continuous IV infusion (Donny et al. 2003) or acute SC injection (Barrett and Bevins 2013; Barrett and Odum 2011; Olausson et al. 2004a; Olausson et al. 2004b; Raiff and Dallery 2006; Tronci et al. 2010). However, few if any animal studies have sought to replicate *steady-state* circulating levels of nicotine that are typical of daily cigarette smokers, i.e., 15–20 ng/ml (Lawson et al. 1998). The lowest effective doses that have been reported to enhance sensory reinforcers appear to be 0.12 mg/kg/h IV (Liu et al. 2007) and 0.175 mg/kg SC (Olausson et al. 2004a), and even these relatively low doses would be expected to produce high sustained plasma levels of 20–55 ng/ml (LeSage et al. 2002; Turner 1975) and 45–100 ng/ml (Reavill et al. 1990; Turner 1975), respectively.

The present study therefore sought to determine whether nicotine produces unambiguous enhancement of a sensory reinforcer in adult rats exposed to steady-state plasma levels comparable to those found in typical cigarette smokers. Experiment 1 comprised a preliminary investigation of nicotine effects using several different visual reinforcers. Experiment 2 served to compare different nicotine doses, across three modes of administration: intermittent “puff-size” IV infusions, continuous IV infusion, and SC administration. In a final step, minimally effective doses of nicotine were given, in order to measure serum nicotine concentrations.

Methods

Animals

Male Long-Evans rats (Charles River, St. Constant, QC, Canada) initially weighed 264–386 and 248–322 g (Experiments 1 and 2, respectively). Our decision to use only male rats was based on two considerations: (1) we wished to compare as closely as possible with the published literature, which is largely based on male rats, and (2) there is only mixed evidence for sex differences in nicotine-induced reinforcement enhancement in rats, with both positive (Chaudhri et al. 2005) and negative findings (Barrett et al. 2016; Quick et al. 2014). The rats were housed 2–3 per cage in a temperature- and humidity-controlled animal colony maintained on a reverse 12:12 light/dark cycle, with lights off at 0700 h. Rats were handled for 3–4 days before testing. All behavioral testing took place during the dark phase of the cycle. Food and water were available ad libitum, except during test sessions, which occurred between 0800 h and 1700 h. Subjects in Experiment 1 had previously taken part in an unrelated study of food reward (chocolate pellet) anticipation; during this earlier study, no drugs were administered and the rats were tested in similar operant cages in which the two levers were permanently retracted. All experimental protocols were approved by the McGill Medical Faculty Animal Care Committee, in accordance with Canadian Council on Animal Care guidelines, in order to minimize pain and discomfort.

Drugs

Drugs and suppliers were (–)-nicotine hydrogen tartrate salt (Sigma-Aldrich, Oakville, ON), ketamine HCl (Vetalar™, VetrepHarm, London, ON), xylazine HCl (Anased™, Novopharm, Toronto, ON), and also carprofen (Rimadyl™), enrofloxacin (Baytril™) and propofol (1% injectable solution, Diprivan™), all from CDMV, St. Hyacinthe, Quebec. Drugs were dissolved in sterile 0.9% saline as needed. (–)-Nicotine hydrogen tartrate salt solutions were adjusted to pH 7.1–7.3 with NaOH, and stored in aliquots at –20 °C until the day of

use. For IV nicotine administration, a stock solution (40 mg/ml) was prepared; delivery parameters are described under Experiment 2. Nicotine doses are expressed as the base.

Intravenous catheterization surgery, maintenance and verification of catheter patency

Rats in Experiment 2 were implanted with IV silastic catheters (ID 0.51 mm OD 0.94 mm, Fisher Scientific, Montreal, QC) in the right jugular vein, under general anesthesia (ketamine 80 mg/kg IP and xylazine 16 mg/kg IP). The catheter was secured to the vein with silk sutures and was passed subcutaneously to the top of the skull where it was connected to a modified cannula (C313G-5UP; PlasticsOne, Roanoke, VA) mounted to the skull with jeweler's screws and dental cement. The analgesic carprofen (5 mg/kg SC) was administered during surgery to alleviate post-surgical pain. Immediately post-surgery, catheters were flushed with 0.1 ml of a sterile solution of heparin (0.2 mg/ml), the antibiotic enrofloxacin (15 mg/kg, Baytril™), and saline. The cannula was occluded with a plastic stopper (Tygon tubing, Fisher Scientific, Montreal, QC) and shielded with an aluminum cap when not in use. Catheters remained unopened during the 8-day post-surgery recovery period.

From the start of behavioral testing, catheters were flushed on alternating days with 0.1 ml of sterile saline or a solution of heparin (0.2 mg/ml), enrofloxacin (15 mg/kg, Baytril™), and saline. In addition, catheter patency was occasionally assessed, where needed, by rapid (3–4 s) IV injection of the general anesthetic propofol (7 mg/kg, i.e., approx. 0.3 ml of a 1% solution). This dose produces an immediate loss of righting reflex followed by recovery within 2–3 min.

Behavioral apparatus and testing procedure

Subjects were tested in operant conditioning chambers (ENV-008CT, Med Associates, Lafayette, IN) housed within melamine cubicles. Each box was equipped with two retractable levers (ENV-112CM) located 10 cm apart and 8 cm above the stainless steel bar floor. A white cue light (2.5 cm diameter, 28 V, 100 mA, ENV-221 M) was situated 3 cm above each of the two levers. A white house light (28 V, 100 mA, ENV-215 M) was located on the opposite wall; this was only used in Experiment 1. All visual stimuli were controlled by Med Associates software. For each rat, one lever was designated “active” and the other “inactive”. The left-right positions of the active and inactive levers were counterbalanced within each group of subjects. An FR1 schedule of reinforcement was used, with a single response on (only) the active lever producing a visual stimulus. The stimulus comprised either a cue light illuminated above the active lever (both Experiments), or a house light (Experiment 1 only). A response on either lever resulted in the immediate retraction of both levers for a time-out period of

60 s, after which the levers were again extended into the chamber. Hence, rats could obtain almost 60 visual stimuli per 60-min session at maximum.

Nicotine (or saline) was either injected SC immediately before the test session, or given IV over the course of the test session. Drug was always administered independently of the animals' behavior, i.e., not self-administered. Intravenous solutions were delivered via liquid swivels (rodent swivel model RSP1, Lomir, Notre-Dame-de-L'Île Perrot, Québec), which were connected to a 3 or 20 ml plastic syringe mounted in a variable rate infusion pump (Med Associates PHM-107). For continuous infusion, the pumps were set to deliver 1.32 ml in 60 min. For intermittent IV delivery, 33 pulses (each of 0.11 ml, 3-s or 30-s duration) were given at constant intervals of 109 s throughout the 60-min session, at doses shown in Table 1. Delivery volumes were confirmed by weighing multiple fluid deliveries with no rat connected.

Serum nicotine assay

Subjects were profoundly anesthetized with Na pentobarbital (Somnotol) 70 mg/kg IP, several minutes before sacrifice. Trunk blood was collected after decapitation. Samples were centrifuged at 3000g for 10 min at 4 °C in order to isolate the

Table 1 Timelines for experiments 1 and 2

Days	Nicotine (mg/kg per session)	Delivery
Experiment 1		
1–4	0 (i.e., saline)	SC
5–18	0 and 0.1	SC
Experiment 2		
1–4	0 (i.e., saline)	SC
5–8	0 and 0.1	SC
9–16	0 ¹ , 0.025, 0.05, 0.1, 0.2 0 and 0.1	IV continuous ² SC
17–22	0, 0.05, 0.1, 0.2 (0.0015–0.006/pulse) ³ 0 and 0.12 ⁴	IV pulses (33 total, 30-s) IV continuous
23–28	0 and 0.1 (0.003/pulse) 0 and 0.1 (0.003/pulse) 0 and 0.1	IV pulses (33 total, 3-s) IV pulses (33 total, 30-s) IV continuous
29–34	0 ¹ , 0.025, 0.05, 0.1, 0.2	SC
35–36	0, 0.05, and 0.1 0, 0.05, and 0.1	SC IV continuous

Notes:

1. The saline condition was tested twice in these dose-response blocks
2. IV continuous delivery refers to constant-rate infusion throughout the 60-min session
3. Nicotine was given as thirty-three 30-s IV pulses, occurring at constant intervals throughout the session (see Methods). The dose per pulse was varied across sessions
4. Dose miscalculation: the intended dose was 0.10 not 0.12 mg/kg

upper transparent serum layer. Serum samples were frozen at -80°C and later assayed for nicotine content in the laboratory of Dr. Paul Pentel (University of Minnesota). This assay is based on gas chromatography with nitrogen-phosphorus detection and has a detection limit of approx. 2 ng per 1 ml sample (Harris et al. 2010).

Experiment 1: repeated nicotine tests with different visual stimuli

This initial experiment sought to determine (1) whether the visual stimuli were reinforcing, (2) whether nicotine selectively enhanced active lever responding, and (3) whether this effect emerged upon repeated testing. To this end, 29 rats were tested in 18 daily 60-min behavioral sessions (weekends excepted), randomly assigned to four groups. After four saline sessions (i.e., days 1–4), each rat was tested alternately with nicotine (0.1 mg/kg SC) and saline (Table 1). The order of drug testing (i.e., NSNS... vs. SNSN...) was counterbalanced across rats. Responding on the active lever resulted in one of four types of visual stimulus, depending on group: 3-s continuous cue light, 3-s flashing cue light, 60-s flashing cue light, and 60-s flashing house light ($n = 7\text{--}8$ rats per group). Flashing stimuli were delivered at 5 Hz, in cycles of 0.1 s on and 0.1 off.

Experiment 2: repeated nicotine tests: dose, mode of delivery, and serum levels

The purpose was to determine (1) whether nicotine exerts a reinforcement enhancing effect at low doses that provide serum levels typically associated with cigarette smoking, and (2) whether this behavioral effect is altered by pulsatile drug delivery. In this experiment, responding on the active lever illuminated a cue light continuously for 3 s. The findings of Experiment 1 suggested that repeated nicotine exposure might hasten the emergence of a reinforcement enhancing effect; to this end, rats were given home cage once-daily injections of nicotine (0.1 mg/kg SC) on 4 consecutive days just prior to surgery.

Twenty-nine rats were tested in 34 daily 60-min behavioral sessions (weekends excepted). The experiment was divided into several testing blocks (Table 1). Days 1–4 comprised saline test sessions, which were followed 1–3 h later by a home cage injection of nicotine (0.1 mg/kg SC). On days 1 and 2 only, for the purpose of habituation, rats were briefly connected to the catheter tubing and then disconnected before injection. Procedural details for each subsequent block are given in Table 1. The order of drug testing was either counterbalanced (days 5–8), randomized (days 9–16), or followed incomplete Williams square designs in order to balance first order carry-over effects (days 17–22, 23–28, 29–34). After each testing block, rats were tested for catheter patency as described above.

Finally, rats were sacrificed for blood collection (days 35–36). To this end, subjects were randomly allocated to receive nicotine, delivered either SC (0, 0.05, or 0.1 mg/kg) or by continuous IV infusion (0, 0.05, or 0.1 mg/kg/h). Sacrifice was timed to coincide with the maximal behavioral effect of nicotine, i.e., 25 min after SC injection (see days 29–34) or 50 min after the start of IV infusion (see days 9–16, 17–22).

Data analysis and statistics

As detailed above, subjects were assigned to groups and conditions in a randomized or counterbalanced manner. Experimenters were not blind to drug conditions. Commercial software was used for statistical analyses (SYSTAT version 11, SPSS Inc., Chicago, IL, USA). The dependent variables were the number of active and inactive lever presses per 10-min time bin or per 60-min session. The number of reinforcers earned was identical to the number of active presses, and hence was not analyzed. Data were subjected to repeated measures analysis of variance (ANOVA) with one or more of the following within-subject factors: NIC (nicotine vs. saline), DOSE, PULSE (3-s pulses vs. 30-s pulses vs. continuous infusion), DAY (i.e., pairs of sessions in Experiment 1 and individual sessions in Experiment 2), and TIME (time within session). The rates of active and inactive lever responding were not directly compared by ANOVA, because this would have violated the homoscedasticity assumption underlying this test. For repeated measures ANOVA, the Huynh-Feldt sphericity-corrected p value is reported. The only between-subject factor was STIMULUS (i.e., the four visual stimulus conditions in Experiment 1). Multiple pairwise comparisons were made using paired t tests with p values subjected to the Holm-Bonferroni adjustment (Ludbrook 1998), referred to here as “H-B paired t -tests”. Active vs. inactive lever preference was assessed in each individual rat using a paired t test, with session serving as the experimental unit. The nicotine serum data were subjected to Kitchen’s BO test (Kitchen 1987). All p values refer to 2-tailed tests, and $p < 0.05$ was considered statistically significant.

Results

Experiment 1 repeated nicotine tests with different visual stimuli

The four types of visual stimulus could not be differentiated statistically, either in terms of the STIMULUS main effect or interactions. Results are therefore presented for the pooled groups (i.e., $n = 29$ rats; Fig. 1). Preferential responding on the active lever was evident from the first (saline) test onwards. Taking the period of drug testing (days 5–18) as a whole, nicotine increased active lever responses (NIC $F_{1, 25} = 15.67$,

Fig. 1 Experiment 1: Enhancement of active lever pressing by nicotine (0.1 mg/kg SC) emerged over days of testing. Four saline tests (S1 to S4) were followed by 7 pairs of alternating saline and nicotine tests (P1 to P7). The y-axis shows the mean $\pm$ SEM active and inactive lever responses per 60-min session ($n = 29$ rats). * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$, nicotine vs. corresponding saline condition (Holm-Bonferroni adjusted paired t tests)

$p < 0.001$). This stimulant effect was not evident in drug-naïve animals (days 5 and 6) but emerged over successive nicotine and saline tests (i.e., days 5–18: NIC $\times$ linear trend on DAY $F_{1, 25} = 8.90$, $p < 0.01$). Nicotine did not significantly alter inactive lever responses (NIC $F_{1, 25} = 1.52$, NS) and no stimulant effect on inactive lever responding emerged over successive tests (NIC $\times$ linear DAY $F_{1, 25} = 3.57$, NS).

Experiment 2 repeated nicotine tests: dose, mode of delivery, and serum levels

General Across the 34 days of the experiment, only 1 of the 29 rats failed to show a significant preference for the active lever (H-B paired t tests, $n = 34$ sessions); this rat was therefore excluded from all analyses. The remaining 28 subjects maintained active lever responding throughout the experiment, and the daily group mean varied from about 10 to 20 active lever responses per 1-h session. Inactive lever responses declined over successive days (linear trend on DAY: $F_{1, 27} = 51.65$, $p < 0.0001$). Both active and inactive lever responding declined reliably within individual 60-min sessions; main effects of TIME (i.e., time within session) are not reported but are illustrated in the figures.

Days 1–4: tests with saline The active lever was preferred on each day, including the first session (active vs. inactive presses, H-B paired t tests: $t_{27} = 3.26$ – 5.36 , $p = 0.0030$ – 0.0001). Pooled across days 1–4, active and inactive lever responses per session were, respectively, 14.4 ± 1.0 and 8.2 ± 0.6 (mean $\pm$ SEM, $n = 28$ rats).

Days 1–8: initial tests with subcutaneous nicotine Nicotine significantly increased active lever responses (H-B paired t test: $t_{27} = 5.48$, $p < 0.0001$) but not inactive lever responses ($t_{27} = 1.80$, NS). Pooled across days 5–8, active lever

responses for saline and nicotine sessions were, respectively, 11.1 ± 0.8 and 17.3 ± 1.4 ($n = 28$). Corresponding values for inactive lever responses were 4.3 ± 0.6 and 5.2 ± 0.6 . An increase in active lever pressing was evident even upon the first nicotine test (H-B paired t test: $t_{27} = 5.55$, $p < 0.0001$), presumably reflecting the fact that in this experiment, rats had already received several home-cage nicotine injections.

Days 9–16: continuous intravenous nicotine dose-response

Results for days 9–16 are shown in Fig. 2 (panels A, B and C). Intravenous nicotine administration significantly increased active lever responses in a dose- and time-dependent manner (DOSE $F_{4, 108} = 17.50$, $p < 0.0001$; DOSE $\times$ TIME $F_{20, 540} = 4.11$, $p < 0.0001$). Inactive lever responses also appeared affected in a time-dependent way (DOSE $F_{4, 108} = 3.13$, $p < 0.05$; DOSE $\times$ TIME $F_{20, 540} = 1.81$, $p < 0.05$). Active lever pressing was stimulated most strongly in the final 20 min of the session (Fig. 2B), with significant increases at 0.1 and 0.2 mg/kg (H-B paired t tests: $p < 0.0001$ for both). Inactive lever responses also appeared increased at this time (DOSE $F_{4, 108} = 4.45$, $p < 0.01$), with an apparent effect at 0.2 mg/kg which did not, however, survive correction for multiple comparisons ($p = 0.051$; Fig. 2C).

Subcutaneous administration of nicotine (0.1 mg/kg) increased active lever responses (Fig. 2A; $F_{1, 27} = 8.77$, $p < 0.01$) and this effect appeared time-independent (NIC $\times$ TIME $F_{5, 135} = 1.02$, NS). The time course of responding (not shown) was similar to that shown on days 29–34 (Fig. 3E below), except that there was no clear effect in the first 10-min time bin ($t_{27} = 1.76$, NS). Inactive lever responses were not significantly altered by SC nicotine ($F_{1, 27} = 0.17$, NS; Fig. 2A).

Days 17–22: pulsed intravenous nicotine dose-response

Results for days 17–22 are shown in Fig. 2 (panels D, E, and F). Nicotine (0, 0.05, 0.1, and 0.2 mg/kg/session), delivered in a series of 30-s IV pulses, increased active lever responses in a dose- and time-dependent manner (DOSE $F_{3, 81} = 31.50$, $p < 0.0001$; DOSE $\times$ TIME $F_{15, 405} = 3.40$, $p < 0.0001$). Pulsed nicotine also affected inactive lever responses, but in a subtle time-dependent way (DOSE $F_{3, 81} = 2.23$, NS; DOSE $\times$ TIME $F_{15, 405} = 2.22$, $p < 0.05$).

For the session as a whole, pulsed nicotine significantly increased active lever responses at 0.1 and 0.2 mg/kg/session, with no significant effect on inactive lever responses (Fig. 2D). Continuous nicotine infusion (tested only at 0.1 mg/kg) also increased active but not inactive lever responses (Fig. 2D). Note: through a miscalculation, the continuous infusion dose was 20% higher than intended (i.e., 0.12 mg/kg instead of 0.10 mg/kg for days 17–22).

Pulsed nicotine appeared to increase active lever responses maximally in the final 20 min of the session (Fig. 2E), with significant effects again at 0.1 and 0.2 mg/kg (H-B paired t

Fig. 2 Experiment 2 (days 9–16 and days 17–22): Continuous and pulsed intravenous nicotine administration both dose-dependently increased active lever responding. *Left panels* (days 9–16): Nicotine was given either as a continuous IV infusion (doses expressed as mg/kg per 60-min session) or as an acute SC injection (0.1 mg/kg) immediately pre-session. *Right panels* (days 17–22): Nicotine was given as a series of 30-s IV pulses, or as a continuous infusion of 0.12 mg/kg/session (20% higher dose than intended, see Table 1). Each rat was tested under all conditions. Panels A and D show total-session active and inactive lever responses. Panels B, C, E and F show the time course of active or inactive lever responses, as indicated; symbols for each of the bottom panels are shown in the overlying panel. For clarity, data relating to SC administration are omitted from panels B and C. Y-axes show mean $\pm$ SEM, $n = 28$ rats. * $p < 0.05$ and *** $p < 0.001$ vs. zero-dose condition (Holm-Bonferroni adjusted paired t tests)

tests: $p < 0.001$ and 0.0001 , respectively). Within this time period, mean $\pm$ SEM active lever responses per 10 min at 0, 0.05, 0.1 and 0.2 mg/kg were respectively 1.8 ± 0.3 , 2.3 ± 0.3 , 2.7 ± 0.4 and 4.6 ± 0.4 . During the same period, inactive lever responses were unaffected (Fig. 2F).

Days 23–28: 3-s vs. 30-s pulses vs. continuous nicotine As shown in Fig. 3A, nicotine increased active lever responses to a comparable extent in the three infusion conditions (NIC $F_{1, 27} = 34.97$, $p < 0.0001$; PULSE $\times$ NIC $F_{2, 54} = 0.055$, NS). The 3-way interaction of PULSE $\times$ NIC $\times$ TIME was also statistically significant ($F_{10, 270} = 2.43$, $p < 0.05$) but difficult to discern (Fig. 3B). Inactive lever responses (Fig. 3C) were unaffected by nicotine overall (NIC $F_{1, 27} = 2.30$, NS), but there was a significant NIC $\times$ TIME interaction ($F_{5, 135} = 4.23$, $p < 0.01$). In the final 20 min, where stimulation of active lever responses appeared maximal, nicotine did not significantly increase inactive lever responding in any of the three delivery conditions (H-B paired t tests, all NS).

Days 29–34: subcutaneous nicotine dose-response Nicotine increased active lever responses (Fig. 3D: DOSE $F_{4, 108} = 19.21$,

$p < 0.0001$), with significant effects at 0.1 and 0.2 mg/kg (Fig. 3E). Inactive lever responses were unaltered (DOSE $F_{4, 108} = 1.01$, NS; Fig. 3F). Active lever responding was increased by nicotine throughout the session (Fig. 3E), but in a time-dependent way (DOSE $\times$ TIME $F_{20, 540} = 2.08$, $p < 0.01$), such that the maximal effect appeared to occur 10–40 min post-injection (i.e., time bins 2–4). In a post hoc analysis of data collapsed across this time interval, nicotine significantly increased active lever responses at 0.05 mg/kg as well as at the higher doses (H-B paired t tests: $p < 0.05$ – 0.0001).

Days 35–36: serum nicotine levels For a given dose of nicotine, IV and SC administration resulted in closely comparable serum concentrations (sampled at 25 and 50 min post-injection, respectively, corresponding to the time of maximal behavioral effect). The lower dose of nicotine (0.05 mg/kg IV and SC) provided 11.5 ± 0.4 and 12.4 ± 0.8 ng/ml, respectively, whereas the higher dose (0.1 mg/kg IV and SC) provided 23.8 ± 0.5 and 26.5 ± 1.0 ng/ml, respectively (mean $\pm$ SEM, $n = 5$ – 6). As expected, nicotine was undetectable in serum from the five saline-injected rats.

Fig. 3 Experiment 2 (days 23–28 and days 29–34): Different types of nicotine pulses produced comparable and selective increases in active lever responding. *Left panels (days 23–28)*: Saline or nicotine (0.1 mg/kg per 60-min session) was delivered either as a series of equally spaced short (3-s) or long (30-s) pulses, or by continuous (Cont) infusion (further details in Table 1). *Right panels*: Panels A and D show active and inactive lever responses occurring during the whole 60-min session. Panels B, C, E, and F show the time course of active or inactive lever responses, as indicated; symbols for each of the bottom panels are shown in the overlying panel. Y-axes show mean $\pm$ SEM, $n = 28$ rats. Each rat was tested under all conditions. *** $p < 0.001$ vs. zero-dose condition (Holm-Bonferroni adjusted paired t tests)

Discussion

In the present study, acute nicotine administration enhanced the effectiveness of a primary sensory reinforcer in a behaviorally specific manner, over several weeks of testing. We show for the first time that this reinforcement enhancing effect can occur at doses low enough to reproduce steady-state serum nicotine levels typical of habitual cigarette smokers. Furthermore, we show that the temporal pattern of drug delivery was unimportant, in that for a given total dose, subcutaneous injection and continuous drug infusion were both as effective as repeated pulsed delivery. Finally, reinforcement enhancement appeared to track serum nicotine levels across the test session.

Methodological considerations

Our behavioral procedures differed in several respects from the protocols used by several other groups. First, the operant procedure combined an FR1 schedule with lever retraction

during time-out periods. In this way, all active lever presses were reinforced, and no opportunity was provided for response extinction during timeout periods. Second, our rats were neither food-restricted nor trained in advance to lever-press for food, whereas in many previous studies rats have been subjected to chronic food restriction and first trained to respond for food reinforcers with subsequent substitution of a visual stimulus (e.g., Barrett and Odum 2011; Caggiula et al. 2002; Chaudhri et al. 2005; Jones et al. 2010; Palmatier et al. 2006; Swalve et al. 2015; Tronci et al. 2010). Food restriction and training tend to promote responding in the initial light-reinforced sessions (Rupprecht et al. 2015) but potentially complicate data interpretation. Third, the visual stimulus (3-s cue light) used in the main experiment (Experiment 2) was probably less reinforcing than the compound visual stimuli (e.g., light on/house light off) common in previous studies (e.g., Caggiula et al. 2002). Despite these differences, our rats responded preferentially on the active lever from the first test session onwards, and almost all subjects maintained a clear preference for the active lever across the several weeks of

testing, as previously reported with a stronger visual reinforcer (Weaver et al. 2012).

Our light stimulus could clearly serve as a primary reinforcer, as shown by tests in drug-naïve subjects (Experiment 1). This visual stimulus is unlikely to have acquired significant secondary reinforcer properties through subsequent association with nicotine, for several reasons: (1) within-session nicotine delivery and stimulus presentation were not explicitly paired, but rather occurred independently, (2) nicotine and saline test sessions were equally numerous, and (3) in Experiment 2, additional nicotine injections occurred before any behavioral testing commenced and also after the initial test sessions.

Nicotine's ability to enhance primary sensory reinforcers emerges with repeated drug testing (Palmatier et al. 2007b; Wing and Shoaib 2010). Accordingly, in Experiment 1, a reinforcer-enhancing effect of nicotine emerged only after several test sessions; since these rats were initially drug-naïve, a stimulant effect may have been masked by nicotine's widely reported behavioral depressant effects (for review, see Clarke 1987). Therefore, to avoid this complication, rats in Experiment 2 were first given several home cage injections of nicotine; reinforcer enhancement now occurred even within the first test session. Thus, as previously demonstrated for a conditioned reinforcer (Olausson et al. 2004b), enhancement of the primary visual reinforcer did not require repeated *testing* under drug.

Behavioral selectivity of nicotine-induced reinforcement enhancement

In drug-experienced rats, nicotine appears to exert a rather general stimulant effect upon behavior. Thus, nicotine not only reliably increases locomotor activity (Clarke 1987), but can also increase low-rate operant responding (Corrigall et al. 1988), even during timeout periods (Chaudhri et al. 2007; Clarke and Kumar 1983) and on a DRL (differential low rate of reinforcement) schedule (Morrison 1968). The present study therefore examined inactive lever responding in some detail. Since unreinforced response rates tended to be low and somewhat variable, we used large sample sizes ($n \sim 30$ rats) and repeated measures designs in order to improve statistical power. We tested multiple nicotine doses, routes and delivery parameters, and paid particular attention to conditions where active lever responding was maximally increased by the drug. The present findings, taken together, show that nicotine *specifically* increased active lever pressing at a dose of 0.1 mg/kg and possibly lower. Even at the highest dose tested (0.2 mg/kg/session IV or SC), nicotine repeatedly failed to detectably increase inactive lever pressing, with one possible exception (Experiment 2 days 9–16, $p = 0.051$ after correction for multiple comparisons). The latter result weakly suggests that high doses of nicotine might be potentially less behaviorally selective than low doses, but against this, there are multiple reports of lever-selective effects at higher doses:

0.35 mg/kg SC (Olausson et al. 2004a, b), 0.4 mg/kg SC (Weaver et al. 2012), 0.48 mg/kg/h IV (Liu et al. 2007), and even 0.99 mg/kg/h IV (Chaudhri et al. 2007). Overall, therefore, nicotine dose cannot readily explain why the drug enhanced only active lever responding in some published studies but not in others.

Reinforcement-enhancing effects of nicotine appear to track plasma and brain levels

To our knowledge, there are no pre-existing studies that directly relate reinforcement enhancement to measured levels of blood nicotine. Our serum levels of nicotine are broadly consistent with literature reports based on SC or IV administration in adult rats (Harris et al. 2010; Pratt et al. 1983; Turner 1975). After SC injection, plasma and brain nicotine levels peak within 5–10 min of injection, and then decline slowly over the next hour (Ghosheh et al. 1999; Hirschhorn and Rosecrans 1974; Pratt et al. 1983; Rollema et al. 2010; Tripathi et al. 1982; Turner 1975). In contrast, our continuous or intermittent IV infusions would be expected to produce steadily *increasing* nicotine levels across the 1-h session (Guillem et al. 2005; LeSage et al. 2002; Turner 1975). The present behavioral data correspond quite closely to these pharmacokinetic patterns. Thus, when nicotine was given subcutaneously, reinforcement-enhancing effects appeared rapidly and tended to be sustained across the session (Fig. 3E), whereas during IV infusions, the drug effect tended to grow across the 60-min session (Figs. 2B, E, and 3B). Hence, reinforcement enhancement by nicotine appears correlated with the concurrent drug concentration. Another potential explanation for nicotine's behavioral time course would be that nicotine slows habituation to the visual reinforcer, but in a parallel study we were unable to detect such an effect (Wright et al., submitted).

Reinforcement enhancement vs. smoking nicotine levels

In the present study, reinforcement enhancement became detectable at doses as low as 0.1 mg/kg IV, and 0.05–0.1 mg/kg SC, whereas previous attempts to detect effects in this dose range have not shown a convincingly selective effect on active lever responding (Barrett and Odum 2011; Wing and Shoaib 2010). To our knowledge, the minimal doses previously shown to enhance sensory reinforcers were 0.175 mg/kg SC (Olausson et al. 2004a) and 0.12 mg/kg IV (Liu et al. 2007). In Experiment 2, reinforcement enhancement was repeatedly seen at 0.1 mg/kg (SC and IV), with post hoc analysis suggesting a possible effect even at the lower dose of 0.05 mg/kg SC. These two doses provided serum nicotine levels of around 12 and 25 ng/ml, respectively, at the time of maximal behavioral effect. This range compares closely to the experience of an average daily smoker, who currently consumes approximately 15 cigarettes per day (Hughes and Callas 2010) and

achieves trough (i.e., between-cigarette) venous nicotine concentrations of 15–20 ng/ml (Lawson et al. 1998).

Reinforcement-enhancement by nicotine was obtained not only with rapid bolus IV infusions, but for the first time with “puff-size” nicotine doses each infused over 30 s, hence more closely modeling the kinetics of uptake from the lungs (Rose et al. 1999). Continuous IV infusion appeared at least as effective as pulsatile delivery, consistent with a previous report (Donny et al. 2003). Whether these different modes of nicotine administration all produce reinforcement enhancement via the same neuropharmacological mechanism remains a question for future research.

Rapid bolus delivery of our “puff-size” doses might have proven more effective if infusions had been synchronized with opportunities to lever press. However, cigarette smokers do not experience significant puff-associated nicotine spikes (Rose et al. 1999), and reinforcement enhancement occurs in human subjects even when nicotine is delivered slowly, by lozenge or patch (Barr et al. 2008; Dawkins et al. 2006).

Study strengths and limitations

Study strengths included methodological simplicity, large sample sizes, and within-experiment replication. Study limitations include the following. First, the rats were given nicotine once daily and hence would have cleared the drug more completely between exposures than in cigarette smokers. Second, only male rats were used; however, a sex difference in nicotine-induced reinforcement enhancement (Chaudhri et al. 2005) has not been found in all studies (Barrett et al. 2016; Quick et al. 2014). Third, the main experiment was lengthy, so that over the course of successive testing blocks, rats were gaining in drug history and behavioral experience. Nevertheless, for a given nicotine dose and mode of delivery, similar-sized effects were observed across testing blocks (compare Figs. 2A, D, and 3A, D).

Conclusion

The present findings show for the first time that nicotine can produce behaviorally selective enhancement of a sensory reinforcer at levels of drug exposure comparable to those experienced by smokers. The nicotine dosing regimes established here may be of use in mechanistic studies.

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