ABSTRACT
Attempts to explain tobacco addiction have relied heavily on the assumption that each cigarette puff delivers a bolus of nicotine to the brain within seconds. However, nicotine transits from lungs to brain much more gradually than once thought. Nevertheless, animal self-administration studies continue to use rapid (e.g., <3-s) infusions, as well as high unit doses of nicotine (e.g., 15–30 µg/kg/infusion), each equivalent to one to two cigarettes. Here, we report that nicotine is self-administered across a range of infusion durations (3, 30, 60, and 120 s) in rats. Slow (30-s) infusions were preferred over fast (nominal 3-s) infusions and were self-administered across several reinforcement schedules, at doses as low as 3 µg/kg/infusion, equivalent to one to two puffs. A conventional “fast/high” self-administration procedure (3 s-30 µg/kg/infusion) was then compared with our new “slow/low” procedure (30 s-3 µg/kg/infusion) in rats trained on a progressive ratio schedule and acutely challenged with dopamine receptor antagonists. The D1 antagonist R-(-)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine (SCH 23390) (6–25 µg/kg s.c.) reduced intake in both procedures and in rats self-administering cocaine (0.5 mg/kg/infusion). The D2 antagonist spiperone (3–30 µg/kg s.c.) and sulpiride (5–20 mg/kg i.p.) increased intake of fast/high nicotine and cocaine, but markedly reduced intake of slow/low nicotine. In a final test, in which only infusion speed was varied, an acute spiperone challenge produced the same differential effect on nicotine self-administration. In conclusion, our new slow/low nicotine self-administration procedure, designed to better mimic smoking-associated nicotine intake, is pharmacologically distinct from the conventional fast delivery/high-dose procedure.

The nicotine addiction field has been underpinned by the pervasive belief that nicotine reaches the brain in 7 to 10 s after a single puff of a cigarette and that this rapid “bolus” of nicotine is necessary for tobacco dependence (Russell and Feyerabend, 1978). Recent empirical evidence, however, indicates that arterial concentrations of nicotine rise gradually and do not peak until 20 to 30 s after each cigarette puff (Rose et al., 1999), with brain nicotine concentrations peaking approximately 2 min after a puff (Rose et al., 2006). It is interesting that, when nicotine is delivered intravenously to human subjects at high rates (e.g., via 10-s infusions), it can serve as a reinforcer (Rose et al., 2003; Harvey et al., 2004), but it is often misidentified as cocaine or as another stimulant (Henningfield et al., 1983; Jones et al., 1999). However, a critical review of the literature uncovered no convincing evidence that nicotine must be delivered rapidly to be reinforcing in human subjects (Dar and Frenk, 2007), and according to a recent report, human subjects will self-administer relatively slow (30-s) intravenous infusions of nicotine (Sufoğlu et al., 2008).

Despite the evidence outlined above, researchers studying nicotine self-administration in animals continue to use ultra-rapid (<1–4-s) bolus infusions of nicotine that inadequately represent the kinetics of nicotine obtained from cigarette smoking (Matta et al., 2007). To date, the effect of infusion rate has been studied systematically only in rhesus monkeys (Wakasa et al., 1995). Here, infusions of 6 and 24 s, but not 100 s, were found to support self-administration. Comparable evidence in rats is fragmentary. For example, Valentine et al. (1997) commented informally that infusion durations longer than 2 to 3 s were poorly reinforcing, particularly at low-unit doses, and studies that have reported a failure to obtain nicotine self-administration in adult rats have used slower (6-s) infusions (Belluzzi et al., 2005).

In animal studies, nicotine infusion rates of 15 or 30 µg/kg typically generate maximal responding and are hence widely used (Corrigall and Coen, 1989; Matta et al., 2007). In contrast, cigarette smokers extract only 1 to 3 µg/kg nicotine per
puff, i.e., 10 to 30 μg/kg/cigarette (Matta et al., 2007). Hence, most animal studies have used doses of nicotine that are equivalent, on a microgram per kilogram basis, to one to two cigarettes, delivered intravenously in <1 to 4 s. Significant self-administration of “puff-sized” nicotine doses given by rapid infusion has occasionally been reported in rats (Donny et al., 1995; Valentine et al., 1997; Watkins et al., 1999), but higher unit doses (such as 10 μg/kg) given by rapid infusion have not always supported reliable self-administration (Shoaib et al., 1997; Donny et al., 1998; Villégier et al., 2007).

The present series of experiments had three goals. The first was to examine the effect of varying the infusion duration or dose on the reinforcing value of nicotine. The second aim was to evaluate a new procedure for nicotine self-administration in rats using infusion doses and durations that would more closely mimic the kinetics of nicotine delivery in human cigarette smokers. Third, we sought to determine the effects of dopamine (DA) D1 and D2 receptor antagonists on nicotine self-administration. Here, we directly compared two methods of nicotine delivery: our new “slow/low” procedure (30-s infusions of 3 μg/kg) versus a conventional “fast/high” procedure (3-s infusions of 30 μg/kg).

Materials and Methods

Animals. These experiments were conducted at two different locations (second experiment at Concordia University (Montreal, QC, Canada); the remaining experiments at McGill University, Montreal, QC, Canada). Subjects were 169 experimentally naive male Long-evans rats (Charles River Laboratories, St. Constant, QC, Canada), weighing 300 to 350 g at surgery, and housed in reverse-cycle (lights off 7:00 AM, lights on 7:00 PM), humidity-controlled (50–60%) rooms in the animal facilities. Animals were given food and water ad libitum while in the home cage and housed singly (Concordia) or in groups of three (McGill). All behavioral testing was done during the dark phase of the cycle between 9:00 AM and 5:00 PM daily. All procedures were carried out according to the guidelines of the Canadian Council on Animal Care and were approved by animal ethics committees at both Concordia and McGill universities.

Surgery. Rats were surgically implanted with intravenous silastic catheters in the right jugular vein (Sorge et al., 2005), under ketamine (80 mg/kg)/xylazine (16 mg/kg) general anesthesia, and then were allowed 5 to 7 days for recovery.

Apparatus. All training and testing was conducted in operant chambers (ENV-009CT; MED Associates, Lafayette, IN) in custom-made melamine sound-attenuating cubicles. Each box was equipped with two retractable levers (ENV-112CM; MED Associates) located 10 cm apart and 8 cm above the stainless steel bar floor. The operant chambers were fitted with dual-channel liquid swivels (Instech Laboratories, Plymouth Meeting, PA), which were each connected to one or two 20-ml plastic syringes mounted in variable rate infusion pumps (PHM-107; MED Associates). The pumps were set to deliver approximately 100 μl of drug solution in 3 and 30 s, respectively (unless otherwise noted). We noted that the pumps delivered 73% of the total final volume during the 3-s infusion [final total infusion volume was 5.4 ± 0.4 and 33.2 ± 0.5 s, respectively (mean ± S.E.M. of four rats tested three times with each duration)]. Henceforth, the fast and slow infusions are referred to as 3- and 30-s infusions, respectively. Above each lever (3 cm) was a white stimulus light (ENV-221M; MED Associates) that was lit at the start of the infusion and stayed on for the entire time-out period (30 or 120 s; see below). In exception, in the second experiment, each box contained two pairs of levers situated on opposite sides; each pair consisted of one retractable “active” lever and one stationary “inactive” lever.

General Procedures. After recovery from surgery, rats were placed in operant test chambers and connected to a swirl that was in turn connected to a syringe in a pump outside the sound-attenuating chamber. After a 5-min time-out at the start of the session, the levers entered the box and a cue light was initially illuminated over the active lever for 30 s to signal drug availability. If a response was made on the active lever, the pump was activated and the cue light above the active lever was illuminated for a total of 30 s from the start of the infusion. All rats were initially permitted to self-administer a fixed ratio (FR) 1 (one lever press for one infusion) schedule of reinforcement until intake stabilized (less than 20% variability across days) before being moved to FR5 (five lever presses for one infusion) and/or proportional ratio (PR) schedules. The PR schedule was based on the following equation: 5e(0.2 x infusion number) – 5 (rounded to the nearest integer), resulting in the following successive response requirements: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, etc. Rats were not food-restricted, food-trained, or given drug primers, and they had their paws placed on the active lever only during the first 2 days when no response was made in the first 30 min of the session. All sessions lasted for a total of 120 min, and all experiments used this procedure unless otherwise noted. At the conclusion of the experiment, all rats were sacrificed via infusion of sodium pentobarbital (30 mg/kg).

Drugs. (±)-Nicotine hydrogen tartrate salt (Sigma Chemical Co., St. Louis, MO) was dissolved in sterile 0.9% saline and adjusted to pH 7.1 to 7.3 with NaOH. A stock solution of 1 mg/ml nicotine (expressed as base) was frozen in aliquots, and one aliquot was thawed to prepare fresh drug solution each day. Cocaine HCl (Medisca Pharmaceutique, Montreal, QC, Canada) was dissolved in sterile saline and a stock solution of 25 mg/ml (salt) was refrigerated at 4°C before use (final dose, 0.5 mg/kg/infusion). SCH 23390 HCl (Sigma Chemical Co.) was dissolved in sterile saline and injected subcutaneously. Spiperone [8-[3-(p-fluorobenzoyl)propyl]-1-phenyl-1,3,8-triazaspiro[4.5]decane-4-one; Sigma Chemical Co.] was dissolved in 0.1 ml of 0.1 N tartaric acid for each 10 ml of solution, diluted with sterile saline, and injected subcutaneously. Sulpiride [5-[5-aminosulfonyl-N-[(1-ethyl-2-pyridyldimethyl]-2-methoxybenzamide; Sigma Chemical Co.] was dissolved in a drop of glacial acetic acid, diluted with sterile saline, and injected intraperitoneally. Control solutions for injection consisted of vehicle. Ketamine HCl (Vetalar; Vetrepthrom, London, ON, Canada) and xylazine HCl (Anased; Novopharm, Toronto, ON, Canada) were injected intraperitoneally with doses expressed as base.

Data Analysis. The following statistical tests were used (see Results for details): analysis of variance (ANOVA) with repeated measures, Dunnett’s, Tukey’s, and paired t tests with Bonferroni correction. A two-tailed p value less than 0.05 was considered significant throughout. For percentage of active lever presses, a subject mean was calculated before calculation of a group mean; the results are expressed as mean ± S.E.M.

Optimal Infusion Duration of a Constant Dose of Nicotine. To test the optimal infusion duration sufficient to promote high levels of responding across various schedules of reinforcement, separate groups of rats (n = 8/group) were allowed to self-administer nicotine at infusion durations of 3, 30, 60, or 120 s. Rats were permitted to self-administer nicotine (15 μg/kg/infusion), initially on an FR1 schedule for 7 days, then an FR5 schedule for 5 days, and were finally switched to a PR schedule for 5 days. Although each group received a different nicotine infusion duration (3, 30, 60, or 120 s), a uniform 120-s time-out was imposed. The dose was chosen to maximize responding based on a previous report (O’Dell et al., 2007).

Simultaneous Choice of a Constant Dose of Nicotine Delivered in 3 or 30 s. Based on the results of the above-mentioned experiment, we sought to determine which of the most self-administered infusion durations (3 or 30 s) was preferred. Rats (n = 12) were prepared with dual catheters in the right jugular vein leading, subcutaneously, to two cannulae mounted on the skull. The operant
chambers were fitted with dual-channel liquid swivels connected to two plastic syringes, each driven by a variable rate infusion pump (PHM-107; MED Associates). Each day, the cannulae were connected to the same drug solution.

For only this experiment, there were four levers in the box during self-administration sessions: two were retractable active levers and two were static inactive levers. At the start of the session, the cue lights above each active lever (constant white light or flashing white light) were illuminated for 30 s. When an active lever was depressed, the cue light was illuminated in the predetermined way, and the pump delivered the dose of nicotine (15 μg/kg/infusion) in the required amount of time. Regardless of the active lever activated, the time-out period was 30 s immediately after the response such that further presses during this time on either active lever were recorded, but had no consequence. Further infusions were available as soon as the cue light was turned off and the time-out period was completed. At no time was there a consequence of pressing the inactive lever, although these responses were recorded. The active lever assigned to deliver each infusion duration and the light stimuli associated with the lever and infusion duration were all fully counterbalanced across rats with respect to the location (left versus right, front versus back) within the chamber. Rats were permitted to self-administer on an FR1 schedule of reinforcement during daily 120-min sessions for a total of 14 days.

**Dose Response for 30-s Infusion Duration.** The previous experiment established that responding for the 30-s infusion duration was consistently higher than for 3-s infusions. Therefore, this experiment tested whether rats would self-administer 30-s infusions of nicotine across a range of doses, i.e., 0, 3, 10, 30, and 60 μg/kg/infusion. Accordingly, rats were randomly allocated to groups and trained to self-administer one dose only (n = 5–8/group). Rats were given daily nicotine self-administration sessions for 9 days on FR1, 5 days on FR5, and 5 days on PR. A different group of rats were subsequently tested in the same way with 0, 1, or 3 μg/kg/infusion (n = 4–6/group). In both cases, different groups were trained on each dose of nicotine to avoid exposure to multiple nicotine doses.

**Effect of the D1 DA Receptor Antagonist SCH 23390 on Responding for Cocaine and Nicotine.** Rats self-administered slow infusions of nicotine at doses as low as 3 μg/kg/infusion (see above). In contrast, the standard procedure for nicotine self-administration uses rapid infusions (<1–3 s) of higher nicotine doses (15–30 μg/kg/infusion). We sought to compare this existing procedure to a new procedure using slower (30-s) infusions of nicotine at low doses (3 μg/kg/infusion). Therefore, separate groups of rats were trained to self-administer nicotine, delivered either by the standard fast/high method (3-s infusion of 30 μg/kg/infusion; n = 11) or by our new slow/low method (30-s infusion of 3 μg/kg/infusion; n = 11). A third group was trained to self-administer cocaine (10-s infusion of 0.5 mg/kg/infusion; n = 9) at a dose and infusion duration used previously (Sorge et al., 2005). Rats self-administered their respective drugs on an FR1 schedule for 6 days before being moved directly to a PR schedule. Pilot data in our laboratory showed that rats maintained the same level of responding for nicotine when moved directly to a PR schedule as when moved to FR5 and then PR; thus, all further experiments omitted the FR5 step to save time and to preserve catheter patency.

Rats were tested with each of four doses of SCH 23390 (0, 6.25, 12.5, and 25 μg/kg) in a counterbalanced manner with two antagonist-free PR days in between each test session. Injections of SCH 23390 were given 30 min before the start of the session. Drug delivery timing and doses were chosen based on previous work with nicotine in rats (Corrigall and Coen, 1991).

**Effect of Two D1, DA Receptor Antagonists, Spiperone and Sulpiride, on Responding for Cocaine and Nicotine.** Rats were permitted, as described above, to self-administer cocaine (n = 8), fast/high nicotine (n = 10), or slow/low nicotine (n = 11). As in the previous experiment, rats were given 6 days on FR1 before being moved to a PR schedule of reinforcement. Each rat was tested once with each dose of spiperone (0, 3, 10, and 30 μg/kg) and sulpiride (0, 5, 10, and 20 μg/kg), with two drug-free PR sessions between each test day for a total of eight test days. On each test day, spiperone or sulpiride was administered 60 or 30 min before the session, respectively. Doses of spiperone and sulpiride were presented in random order and spiperone doses and timing were chosen based on previous work with nicotine (Corrigall and Coen, 1991).

**Effect of the DA D2 Receptor Antagonist Spiperone on Responding for a Constant Dose of Nicotine Delivered in 3 or 30 s.** Rats were permitted to self-administer cocaine (n = 10) or nicotine (15 μg/kg/infusion) delivered in 3-s (n = 11) or 30-s infusions (n = 8) for 9 days on an FR1 schedule before being tested every third day with spiperone. Rats were tested with four doses of spiperone (0, 3, 10, and 30 μg/kg) and given 2 FR1 days between each test.

**Results**

**Optimal Infusion Duration of a Constant Dose of Nicotine.** This experiment tested whether an optimal infusion duration existed across various schedules of reinforcement (Fig. 1). Infusion duration significantly affected nicotine intake on both FR5 and PR schedules [main effect of infusion duration, respectively: F(3,28) = 4.21, p < 0.05; F(3,28) = 7.72, p < 0.001] but not on the FR1 schedule. Further analysis revealed that, on the FR5 schedule, intake of 3- and 30-s infusions was comparable, whereas intake of 60-s infusions was significantly lower (p < 0.05). Similarly, on the PR schedule, 30-s infusions were self-administered significantly more than 60- and 120-s infusions (p values <0.05), and intake of 30-s infusions tended to be higher than for 3-s infusions (p = 0.058). On the PR schedule, rats showed greatest selectivity for the active versus inactive lever for the 30-s infusion duration (3 s, 28.2 ± 8.2 versus 12.4 ± 3.8; 30 s, 55.1 ± 19.3 versus 9.3 ± 2.3; 60 s, 15.2 ± 6.2 versus 6.9 ± 2.7; and 120 s, 21.8 ± 6.1 versus 5.6 ± 1.6). The corresponding percentage of responses on the active lever at each infusion duration was, respectively, 66 ± 8, 84 ± 3, 64 ± 8, and 77 ± 4%.

**Simultaneous Choice of a Constant Dose of Nicotine Delivered in 3 or 30 s.** Figure 2 shows the mean number of infusions of nicotine taken for each infusion duration for the 14-day experimental period, starting on the first day of ac-

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**Fig. 1.** Nicotine self-administration (15 μg/kg/infusion) in rats trained to self-administer nicotine in one of four infusion durations (3, 30, 60, or 120 s, with 120-s time-out period for each; n = 8/group). Three reinforcement schedules were used: FR1 for 7 days, FR5 for 5 days, and finally PR for 5 days. The mean ± S.E.M. number of infusions taken during each of the 120-min sessions averaged across the final 3 days of each schedule is shown. *p < 0.05 (Tukey’s test, group differences).
for 9 days, FR5 for 5 days, and finally PR for 5 days. The mean ± S.E.M. number of infusions taken during each of the 120-min sessions averaged 13.0 nicotine and for the active versus the inactive lever (3 s: show a clear preference for the 30-s over the 3-s infusions of schedules [FR5:

experiment were pooled. Nicotine intake was significantly response data for 30-s infusions of nicotine are shown in Fig. 2.1.

across the 14-day experiment revealed a significant main effect on nicotine and for the active versus the inactive lever (3 s: 13.0 ± 3.3 versus 7.6 ± 3.4 responses; 30 s: 16.3 ± 3.6 versus 2.1 ± 1.0 responses) (Fig. 2).

Dose Response for 30-s Infusion Duration. The dosemresponse data for 30-s infusions of nicotine are shown in Fig. 3. After initial injection, the data from the two parts of the experiment were pooled. Nicotine intake was significantly dose-dependent only on FR5 and PR schedules of reinforcement [main effects of dose on FR5: F(5,40) = 7.95, p < 0.001; PR: F(5,40) = 11.42, p < 0.001]. Particularly from the third day of training, the rats show a clear preference for the 30-s over the 3-s infusions of nicotine and for the active versus the inactive lever (3 s: 13.0 ± 3.3 versus 7.6 ± 3.4 responses; 30 s: 16.3 ± 3.6 versus 2.1 ± 1.0 responses) (Fig. 2).

Fig. 2. Nicotine self-administration (15 μg/kg/infusion) in rats trained to simultaneously self-administer two infusion durations of nicotine over 14 days on an FR1 schedule of reinforcement (n = 12). The mean ± S.E.M. number of infusions taken during each of the 120-min sessions is shown.

Fig. 3. Nicotine self-administration in rats trained to self-administer 30-s infusions of nicotine (0, n = 5; 1, n = 5; 3, n = 13; 10, n = 8; 30, n = 8; and 60 μg/kg/infusion, n = 7). Three reinforcement schedules were used: FR1 for 9 days, FR5 for 5 days, and finally PR for 5 days. The mean ± S.E.M. number of infusions taken during each of the 120-min sessions averaged across the final 3 days of each schedule is shown. *, p < 0.05; ***, p < 0.001 (Dunnett’s test versus 0 μg/kg/infusion).

Fig. 4. Self-administration of cocaine (10 s of 0.5 mg/kg/infusion; n = 9), fast/high nicotine (3 s of 30 μg/kg/infusion; n = 11), or slow/low nicotine (30 s of 3 μg/kg/infusion; n = 11) after treatment with the DA D1 receptor antagonist SCH 23390 (subcutaneous). The mean ± S.E.M. number of infusions taken during each of the 120-min PR test sessions is shown. *, p < 0.05; ***, p < 0.001 (Bonferroni corrected paired t tests versus vehicle injection).

quision. Repeated measures ANOVA for infusions revealed a significant main effect of infusion duration [F(1,22) = 4.83, p < 0.05]. Analysis of the active and inactive lever presses across the 14-day experiment revealed a significant main effect of day for active lever presses only [F(13,286) = 2.39, p < 0.01]. Particularly from the third day of training, the rats show a clear preference for the 30-s over the 3-s infusions of nicotine and for the active versus the inactive lever (3 s: 13.0 ± 3.3 versus 7.6 ± 3.4 responses; 30 s: 16.3 ± 3.6 versus 2.1 ± 1.0 responses) (Fig. 2).

On all three schedules, responding was mostly directed to the active lever. The percentage of responses on the active lever at each nicotine dose (0, 1, 3, 10, 30, and 60 μg/kg/infusion) was as follows: for FR1, 62 ± 6, 63 ± 13, 76 ± 3, 83 ± 4, 83 ± 4, and 86 ± 4%; for FR5, 63 ± 5, 61 ± 9, 87 ± 2, 86 ± 2, 89 ± 3, and 90 ± 4%; and for PR, 66 ± 7, 54 ± 14, 82 ± 5, 90 ± 3, 78 ± 4, and 90 ± 3%, respectively. On the FR and PR schedules, active responses predominated significantly at doses of 3 μg/kg and higher (Bonferroni corrected paired t tests, all p values <0.05). Hence, neither the 1 μg/kg dose nor the light alone in the saline condition was significantly reinforcing.

Effect of the D1 DA Receptor Antagonist SCH 23390 on Responding for Cocaine and Nicotine. Figure 4 shows that the D1 DA antagonist SCH 23390 dose-dependently reduced intake of cocaine and nicotine on a PR schedule. The analysis (ANOVA) of the intake data revealed significant main effects of SCH 23390 dose [F(3,84) = 66.00, p < 0.001] and group [F(2,28) = 16.19, p < 0.001]. Although there was a significant SCH 23390 dose by group interaction [F(6,84) = 2.60, p < 0.05], SCH 23390 had a clear depressive effect in all three groups. A similar result was obtained for active responses (main effects and interaction, p values <0.05–0.001). Inactive responses were inhibited by SCH 23390 [dose main effect: F(3,84) = 6.16, p < 0.001], but there was no significant interaction. Percentage of total responding on the active lever in each test session is shown in Table 1.

Effect of Two D2 DA Receptor Antagonists Spiperone and Sulpiride on Responding for Cocaine and Nicotine. Figure 5A shows the effect of the DA D2 receptor antagonist spiperone on intake of cocaine and nicotine. The...
lever pressing for slow/low nicotine (Fig. 5A). There were no significant effects of spiperone on inactive lever pressing in any of the groups, indicating that the increase or decrease in responding was specific to the active lever.

The DA_D2 receptor antagonist sulpiride exerted similar effects on drug intake (Fig. 5B). Here, there was a significant group effect [$F(2,26) = 124.11, p < 0.001$] and interaction [$F(6,78) = 8.75, p < 0.001$] but no main effect of sulpiride dose. However, for active lever pressing, there were significant main effects of sulpiride dose [$F(3,78) = 4.25, p < 0.05$] and group [$F(2,26) = 24.14, p < 0.001$] and a significant interaction [$F(6,78) = 3.25, p < 0.05$]. Sulpiride (at the low and medium dose) significantly increased responding for cocaine and fast/high nicotine, but all sulpiride doses reduced intake and responding for slow/low nicotine. There were no significant effects on inactive responding. The percentage of total responding on the active lever in each test session is shown in Table 1.

The within-session temporal pattern of responding was then examined (Fig. 6). In the absence of antagonist, the nicotine slow/low rats distributed their responding throughout the session (Fig. 6C), whereas the nicotine fast/high group responded much more in the first half of the session (Fig. 6B). For the cocaine and nicotine fast/high groups, the response-enhancing effect of spiperone and sulpiride were predominantly seen late in the session (Fig. 6, A and B). In contrast, responding in the nicotine slow/low group was dramatically reduced by spiperone and sulpiride within the first hour of the session and remained low throughout (Fig. 6C).

**Effect of the DA_D2 Receptor Antagonist Spiperone on Responding for a Constant Dose of Nicotine Delivered in 3 or 30 s.** Figure 7 shows the intake of cocaine and nicotine after treatment with spiperone on an FR1 schedule. The ANOVA revealed significant main effects of spiperone dose [$F(3,78) = 5.46, p < 0.01$] and group [$F(2,26) = 30.24, p < 0.001$] and a significant dose by group interaction [$F(6,78) = 4.75, p < 0.001$] for intake and similarly for active lever responding (all $p$ values <0.01). No effects on inactive lever responding were significant. Subsequent within-group analyses of intake are shown in Fig. 7. For the cocaine, fast and slow nicotine, the percentage of responding on the active levers for the doses of spiperone were, respectively, for 0 $\mu$g/kg, 30 $\pm$ 4, 89 $\pm$ 5, and 80 $\pm$ 4; for 3 $\mu$g/kg, 88 $\pm$ 3, 83 $\pm$ 7, and 83 $\pm$ 7; for 10 $\mu$g/kg, 88 $\pm$ 4, 90 $\pm$ 3, and 90 $\pm$ 5; and for 30 $\mu$g/kg, 91 $\pm$ 3, 90 $\pm$ 5, and 86 $\pm$ 5.

**Discussion**

**Novel Findings.** The present study yielded three novel sets of findings. First, rats preferred slow infusions of nicotine to fast infusions when given a simultaneous choice of the same dose. Second, rats self-administered slow (30-s) infusions of nicotine over a range of unit doses, including a low-dose equivalent to one to two puffs of a cigarette. Third, the effects of DA_D2 receptor antagonists on nicotine intake depended critically on the infusion speed/dose parameters of self-administered nicotine. As discussed below, these observations have implications for past and future nicotine self-administration studies in animals and human subjects.

**Effect of Infusion Rate and Dose on Self-Administration.** The pattern of fast/high nicotine intake was similar to most previous reports using limited access (i.e., 1–2 h) daily

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**Table 1**

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**Figure 5.** Self-administration of cocaine (10 s of 0.5 mg/kg/infusion; $n = 8$), fast/high nicotine (3 s of 30 $\mu$g/kg/infusion; $n = 10$), or slow/low nicotine (30 s of 3 $\mu$g/kg/infusion; $n = 11$) after treatment with the DA_D2 receptor antagonists spiperone (subcutaneous) (A) and sulpiride (intraperitoneal) (B). The mean ± S.E.M. number of infusions taken during each of the 120-min PR test sessions is shown. * $p < 0.05$ (Bonferroni corrected paired t tests versus vehicle injection).
tests, with a reduced level of intake observed after a transition from FR1 to a more demanding ratio (but see Donny et al., 1995; Caggiula et al., 2002; Shram et al., 2008). Likewise, the drug intake we observed in the PR schedule is comparable with previous results (Donny et al., 1999; Chaudhri et al., 2005).

The present experiments showed that slow (30-s) infusions of nicotine were not only preferred over faster (3-s) infusions in a simultaneous choice procedure (Fig. 2) but also generated higher infusion rates in a between-group comparison (Fig. 7, control groups). Both of these findings were obtained with a constant infusion dose of nicotine (15 μg/kg). A previous study showed that rats did not show a preference between high and low doses of nicotine (8–75 μg/kg i.v.) in a simultaneous choice procedure (Manzardo et al., 2002). Here, we show that a preference emerges when the speed of infusion is varied, but the dose remains constant. In the other experiments, the effect of infusion speed could not be directly assessed, because both the dose and speed of nicotine infusions were varied simultaneously, the intent being instead to compare slow/low versus fast/high self-administration procedures.

Strikingly, slow (30-s) infusions of nicotine supported self-administration over a wide range of doses on various schedules of reinforcement (Fig. 3). It is important to note that the 30-s light stimulus alone (and any associated infusion pump noise) was not significantly reinforcing, in that the zero-nicotine dose group did not respond preferentially on the active lever (Fig. 3). Therefore, either 30-s nicotine infusions
were reinforcing in their own right, or they enhanced a latent reinforcing effect of the 30-s light cue (Chaudhri et al., 2006).

To date, only one other study has formally examined the effects of infusion rate on nicotine self-administration in animals (Wakasa et al., 1995). In that study, rhesus monkeys were trained to self-administer fast infusions of nicotine and were then presented with successively slower nicotine infusions. Although faster infusions were self-administered more than slower infusions, interpretation is difficult for two reasons: the more rapid infusions were more familiar, because they had been used in training, and no inactive lever was provided to test goal-directed behavior. The present study avoided these confounds.

The effects of infusion speed have also been studied with respect to intravenous psychostimulant self-administration, but with mixed results (Liu et al., 2005; Crombag et al., 2008). Liu et al. (2005) found that faster cocaine infusions (5 versus 25 or 50 s) generated higher progressive ratio breakpoints, whereas Crombag et al. (2008) reported no clear effect of infusion speed on PR schedule responding for either amphetamine or cocaine (5–100-s infusions). There seem to be no corresponding studies of drug reinforcement per se in human subjects, although in a related study, rapid intravenous infusions of cocaine were rated as more pleasurable and of higher monetary worth than slower infusions (Abreu et al., 2001).

**DA Antagonist Effects on Nicotine Self-Administration.** The effects of DA D1 and D2 antagonists on nicotine intravenous self-administration have been reported in only one study to date (Corrigall and Coen, 1991). In that study, the D1 antagonist SCH 23390 (10 and 30 µg/kg s.c.) depressed FR5 responding not only for nicotine but also for food, with concomitant decreases in locomotor activity. The present study used similar doses of SCH 23390 and also revealed response-inhibitory effects. This inhibition again seemed general, because it occurred across groups (i.e., cocaine, fast and slow nicotine infusions) and affected inactive lever responding.

Systemic administration of the D2 receptor antagonist spiperone has been reported to reduce FR5 responding for nicotine infusions that approached our fast/high condition (Corrigall and Coen, 1991). Here, in contrast, comparable doses of the same antagonist augmented both FR1 and PR responding (FR5 responding was not tested). This apparent discrepancy may be explained by temporal differences. In particular, Corrigall et al. (1991) used a shorter (60-min) test session, and within this time window, we also found that spiperone tended to inhibit rather than enhance self-administration (Fig. 6).

The present experiments revealed that the effects of D2 receptor antagonists are qualitatively different in the two self-administration procedures, i.e., slow/low versus fast/high. Striking differences were observed not only with a FR1 reinforcement schedule, but also with a PR schedule where increased responding is usually interpreted as an increase in motivation (Richardson and Roberts, 1996). It is important that these D2 antagonists did not significantly affect inactive lever pressing and would not be expected to alter locomotor activity at the doses administered (Corrigall and Coen, 1991). Therefore, it would seem that D2 receptor antagonism made rats less motivated to work for slow/low nicotine infusions but increased their motivation to obtain fast/high nicotine.

Although there seems to be no clear explanation for these findings, recent evidence suggests that DA transmission may modulate both rewarding and aversive effects of nicotine, these functions being segregated between shell and core subregions of the nucleus accumbens (Laviolette et al., 2008; Sellings et al., 2008). We therefore speculate that slow/low and fast/high nicotine delivery may differentially affect DA release in these dopaminergic (DAergic) terminal fields.

It would also be tempting to try to compare our results with those from human studies. However, DA antagonists have not been investigated with respect to intravenous nicotine self-administration, and acute tests of smoking behavior in nonschizophrenic patients have provided mixed results, with both increases and decreases in cigarette smoking reported after acute administration of haloperidol (Caskey et al., 1999; Brauer et al., 2001).

**DA Antagonist Effects on Cocaine Self-Administration.** In the present study, the DA D1 receptor antagonist SCH 23390 inhibited progressive ratio responding for cocaine, consistent with previous reports (Hubner and Moreton, 1991; Depoortere et al., 1993). To our knowledge, only one study has previously examined the effect of the D2 antagonist spiperone on progressive ratio responding for cocaine (Hubner and Moreton, 1991). In that study, breakpoints were reduced by spiperone, a result seemingly at odds with the present findings. However, Hubner and Moreton (1991) used substantially different conditions (a higher unit dose, faster infusions, and a different progressive ratio schedule). It is important that our unit dose of cocaine was almost certainly on the ascending limb of the dose-response curve for a PR schedule (Liu et al., 2005), suggesting that D2 antagonism rendered each cocaine infusion more rewarding.

**Similarities Between Cocaine and Fast/High Nicotine.** Our D2 antagonist challenges revealed a concordance between cocaine and the conventional fast/high nicotine self-administration procedure. These observations extend earlier evidence that intravenous nicotine can mimic certain behavioral and neurochemical effects of cocaine, especially when nicotine is administered by rapid infusion (5 versus 25 or 100 s) and in high doses (5 × 50 µg/kg within 10 min) far beyond those experienced by tobacco smokers (Samaha et al., 2005). It is noteworthy that human subjects have identified high doses of intravenous nicotine (20–40 µg/kg given in 10 s) as having psychostimulant-like subjective properties (Henningfield et al., 1983; Jones et al., 1999), and comparable doses of nicotine (also infused in 10 s) are reported to generate high rates of operant responding (Harvey et al., 2004).

In conclusion, the highly influential “bolus hypothesis” of nicotine dependence (Russell and Feyerabend, 1978) was based on the assumption that nicotine is almost instantaneously extracted from the lungs. In contrast, arterial nicotine concentrations climb gradually over 20 to 30 s after each puff (Rose et al., 1999). Recently, slow (30-s) infusions of nicotine were found to be reinforcing in overnight-abstinent smokers; subjects significantly preferred doses of 6 and 10 µg/kg i.v. over placebo, whereas 1.5 µg/kg was selected at chance levels (Sofuoglu et al., 2008). Our procedurally similar nicotine self-administration assay revealed a comparable dose-relationship, suggesting that further cross-species comparisons may be fruitful. Together, these recent findings...
suggest that cigarette smoking may indeed deliver nicotine sufficiently rapidly to exert detectable reinforcing effects. The published literature suggests that DAergic transmission is critical for the reinforcing effects of nicotine in rats. Thus, acute subcutaneous and intravenous nicotine administration increases DA overflow in nucleus accumbens (Benwell ME and Balfour DJ, 1992; Pontieri et al., 1996), and intravenous nicotine self-administration is inhibited by systemic DA antagonists (Corrigall and Coen, 1991), and by DA-depleting lesions (Corrigall et al., 1992). However, all these previous studies used doses of nicotine (typically 0.4–0.8 mg s.c. or 30–50 μg/kg i.v.) and delivery kinetics that are very different from those that would be experienced by smokers. We demonstrate that rats will self-administer nicotine infusions that more closely mimic the pharmacokinetics of cigarette smoking, but the precise role of DAergic and non-DAergic mechanisms in this new behavioral procedure remain to be established. Finally, it is possible that slower infusions of nicotine provide substantially more opportunity for the reinforcement-enhancing effect of nicotine to occur (Chaudhri et al., 2006). Therefore, future studies will be needed to determine the extent to which the self-administration of slow/low nicotine infusions is motivated by a primary reinforcing effect, as opposed to a reinforcement-enhancing effect of the drug.

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References


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