Rapid Delivery of Nicotine Promotes Behavioral Sensitization and Alters Its Neurobiological Impact

Anne-Noël Samaha, Wai-Ying Wendy Yau, Pengwei Yang, and Terry E. Robinson

Background: *Nicotine is highly addictive when it is inhaled from tobacco smoke, whereas nicotine replacement products, which usually deliver nicotine orally or transdermally, rarely lead to addiction. It has been proposed that this is due in part to differences in the rate of nicotine delivery to the brain under the two conditions. However, the mechanism by which rapid nicotine delivery facilitates the transition to addiction is not known. The ability of drugs to alter gene regulation and to produce sensitization has been implicated in addiction. We hypothesized, therefore, that varying the rate of nicotine administration may modulate its ability to elicit this form of plasticity.*

Methods: *Animals were treated with repeated intravenous infusions of nicotine over 5, 25, or 100 sec, and their locomotor responses were monitored over treatment days.*

Results: *We found that increasing the rate of intravenous nicotine infusion potentiated its ability to produce locomotor sensitization, and to induce c-fos and arc mRNA expression in mesocorticolimbic structures.*

Conclusions: *We suggest that rapid administration may increase vulnerability to addiction by altering the neurobiological impact of nicotine and promoting a form of neurobehavioral plasticity (i.e., sensitization) that can lead to pathological incentive motivation for drugs.*

Key Words: Rate of intravenous infusion, locomotor sensitization, immediate early genes, caudate-putamen, nucleus accumbens, addiction

Nicotine, the primary psychoactive component of tobacco (Balfour 1990), is very addictive when it is inhaled in tobacco smoke, whereas nicotine delivered orally or through the skin, rarely leads to addiction (Henningfield and Keenan 1993; Hughes 1989; West et al 2000). It has been suggested that the lower addictive potential of oral or transdermal nicotine derives in part from the slow delivery of the drug to the brain relative to when it is smoked (Henningfield and Keenan 1993; Hughes 1989). It is not understood, however, how increasing the rate of nicotine delivery to the brain increases its addictive potential (Baker et al 2004).

The ability of drugs of abuse to reorganize brain regions involved in reward and incentive motivation, such as the striatum, and brain regions involved in cognitive control and judgment, such as the prefrontal cortex, is thought to facilitate the pursuit of drugs and thereby promote addiction (Hyman and Malenka 2001; Jentsch and Taylor 1999; Nestler 2001; Robinson and Berridge 1993, 2003). In laboratory animals, one behavioral manifestation of these drug-induced neuroadaptations is psychomotor sensitization (Robinson and Berridge 1993, 2000). Psychomotor sensitization has been observed in animals following repeated exposure to a number of addicting drugs, including nicotine (Clarke and Kumar 1983; Ksir et al 1985; Miller et al 2001; Stolerman et al 1973), and the development of sensitization has been linked to an increased susceptibility to drug reward (Horger et al 1990; Lett 1989; Piazza et al 1989, 1990; Vezina 2004), and to relapse following extinction of drug-seeking behavior (De Vries et al 1998). These findings prompted the idea that sensitization-related adaptations play a role in the transition to addiction (Robinson and Berridge 1993, 2003). We hypothesized, therefore, that increasing the rate of nicotine administration may enhance its ability to produce this form of neurobehavioral plasticity. We tested this hypothesis by examining the influence of rate of intravenous infusion on the ability of nicotine to produce psychomotor sensitization. To begin to explore how rate of delivery may alter the neurobiological impact of nicotine we also studied nicotine-induced immediate early gene (IEG) expression in the mesocorticolimbic system.

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Methods and Materials

Experiment 1: Behavioral Sensitization to Single Daily Infusions of Nicotine

Subjects. All procedures were approved by the University of Michigan Committee on the Use and Care of Animals. Female Sprague Dawley rats (Harlan, Indianapolis, Indiana, 224–249g) were individually housed in plastic hanging cages in a climate-controlled colony room maintained on a 14:10 hr light/dark cycle (lights on at 8:00 am).

Catheter Implantation. One week following arrival to the colony room, rats were anesthetized with a mixture of ketamine and xylazine (77:1.5 mg/ml, intraperitoneal [IP], at .1 ml/100 gm of body weight) and instrumented with intravenous (IV) catheters using procedures described previously (Crombag et al 1996; Weeks 1972; Samaha et al 2002). The catheter was inserted into the jugular vein with the tip positioned just above the heart and tunneled subcutaneously to exit between the animal's shoulder blades. Four to 7 days following surgery, catheters were screened for patency by manually injecting .1 ml (IV) of the short-acting barbiturate sodium thiopenthal (20 mg/ml in sterile water). Rats that became ataxic within 5 sec were considered to have patent catheters and transported to a testing room where they were housed for the duration of the experiment. Rats that did not become ataxic were excluded from the study.

Apparatus. The test chambers were circular, opaque plastic buckets 36 cm in height and 25 cm in diameter, with granulated corncob bedding covering the floor. Each chamber was equipped with a photocell-based automated rotometer that recorded quarter, half, and full turns in each direction, in 2-min intervals, using an XT-based personal computer (McFarlane et al 1992). The number of half turns to the left and to the right were

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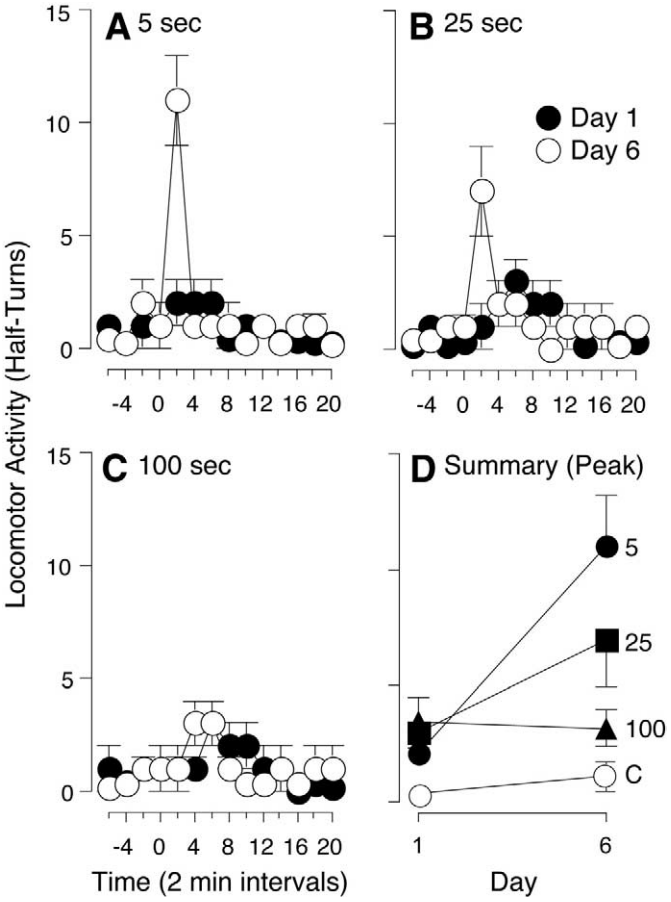

Figure 1. Locomotor activity following an infusion of saline (indicated by "C" for control) or 50 μg/kg nicotine delivered over 5, 25, or 100 sec, on Day 1 and Day 6 of treatment (values = mean ± SEM). **(A–C)** depict the time course of the locomotor response. **(D)** shows the mean peak number of half-turns (averaged over the first 2 min following the infusion), on Day 1 and Day 6.

summed and used as an index of locomotor activity. Prior to each testing session, each rat was tethered to a liquid swivel (Instech Laboratories, Plymouth Meeting, Pennsylvania) via a flexible stainless steel cable. The swivel was fixed on a counter-balanced arm that allowed free movement of the animals in the test chambers. Each animal's catheter was connected to the swivel by a length of Tygon tubing that contained the drug or vehicle solution. A second length of tubing connected the swivel to a pump-driven syringe.

Groups and Procedures. One day after being transferred to the testing chambers, animals were tethered and 40 min later were given an IV infusion of 10 μl of saline to acclimatize them to the testing procedures. The next day, the experimenter filled the infusion lines with 10 μl of saline or 10 μl of a nicotine solution (bitartrate salt, calculated on the weight of the base and dissolved in .9% saline, pH adjusted to 7.2–7.4), connected these to the animals' catheters, and tethered the animals to the swivels with flexible stainless steel cables. The animals were left undisturbed for 40 min during which baseline levels of locomotor activity were monitored. The experimenter then entered the room and activated the pumps so that the drug/saline solution was delivered simultaneously to all animals, regardless of infusion rate. Independent groups of animals received an IV infusion of 25 or 50 μg/kg nicotine over 5 (136.8 μl/min), 25 (24.6 μl/min), or 100 sec (6.84 μl/min), or saline over 5 sec, in a volume of 10 μl. Locomotor activity was recorded for 1 hour following the infusion. This treatment was repeated once a day for 6 consecutive days (Figure 1). At the end of the 6th testing session, animals were disconnected from their tethers and catheter patency was assessed by manual injection of sodium thiopenthal as described above. Only data from animals with patent catheters were used.

Experiment 2: Behavioral Sensitization and Immediate Early Gene (IEG) Expression Following Multiple Daily Infusions of Nicotine

Pilot experiments revealed that a single IV infusion of nicotine (25 or 50 μg/kg) did not increase c-*fos* mRNA expression above control levels, at any infusion rate tested, but that serial infusions of 50 μg/kg nicotine did, and this effect varied with infusion rate. Therefore, in Experiment 2, we examined the influence of infusion rate on the ability of repeated exposure to serial infusions of nicotine to produce behavioral sensitization, and to increase c-*fos* and *arc* mRNA expression. In addition, we examined the influence of infusion rate on nicotine-induced c-*fos* mRNA expression in the two major subpopulations of projection neurons within the caudate-putamen.

Groups and Procedures. Female rats (Harlan, 224–249g) were implanted with jugular catheters as described above. Animals with patent catheters were moved to the circular test chambers described in Experiment 1. One day later animals were given an IV infusion of 10 μl of saline to habituate them to the testing procedures. Beginning on the next day, animals were tethered and 40 min later were given five equally spaced infusions of saline (10 μl each) or nicotine (25 or 50 μg/kg dissolved in 10 μl saline). Each of the five saline infusions was delivered over 5 sec, and each of the nicotine infusions was delivered over 5, 25 or 100 sec. The time interval between individual infusions was adjusted for each infusion rate such that all five infusions were administered within a 10-min period (see Figure 2A). Locomotor activity (the sum of the number of quarter turns to the left and to the right) was recorded for 1 hr following the infusions. This treatment was repeated on 6 consecutive days.

At all infusion rates tested, animals treated with 25 μg/kg nicotine showed no change in their locomotor response to nicotine over testing days, indicating that psychomotor sensitization did not develop at this dose. Moreover, pilot experiments showed that 25 μg/kg given over 5 or 100 sec failed to elicit a greater c-*fos* response than saline in either the core or shell of the accumbens and had only a very marginal effect in the caudate-putamen. Animals treated with this dose of nicotine were therefore excluded from subsequent testing. Animals treated with 50 μg/kg nicotine were left drug-free for four days before receiving one final test session. On this day, all nicotine-treated animals received five infusions of 50 μg/kg nicotine, delivered over the same rate they had received during the pretreatment phase. The saline-treated animals were divided into 4 groups and given five infusions of nicotine over 5, 25 or 100 sec, or saline over 5 sec. Forty minutes after the infusions, animals were disconnected from their tethers and catheter patency was assessed as described above. Animals with patent catheters were immediately taken to a separate room and decapitated. Their brains were shock-frozen in isopentane (−40 to −50° C) and stored at −80° C.

Single In Situ Hybridization. Coronal brains sections (16 μm) were cut on a cryostat at 200 μm intervals from approximately +3.8 mm to −.8 mm relative to bregma. Using a protocol adapted from Cullinan et al (1995), and described in detail by Uslaner et al (2001), brain sections were processed for single in

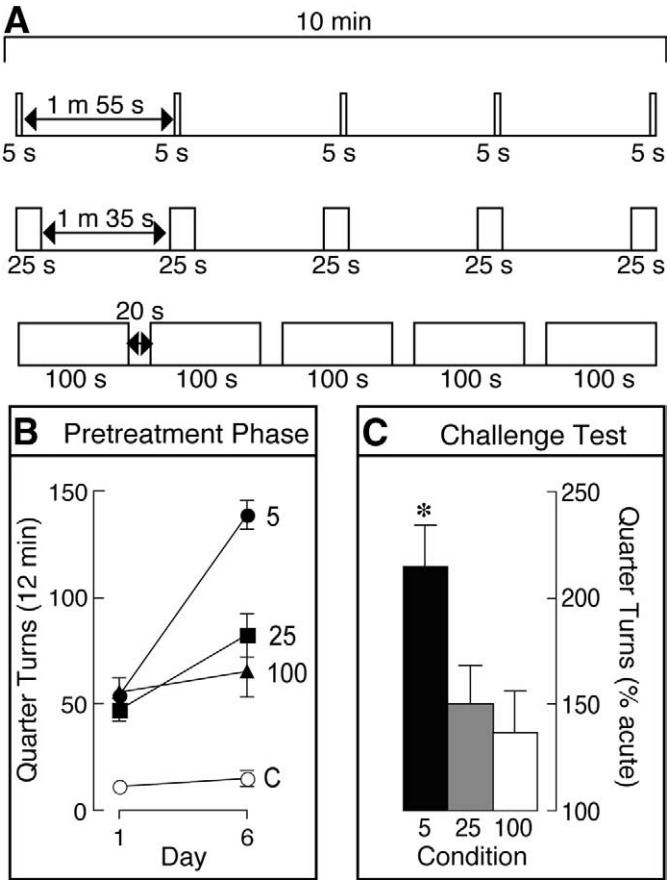

Figure 2. Locomotor activity following serial infusions of saline ("C") or 50 μg/kg nicotine given over 5, 25, or 100 sec, on Day 1 and Day 6 (**B**; values = mean ± SEM), as well as the locomotor response (averaged over the first 12 min following nicotine administration and depicted as percent of that in saline-pretreated animals given nicotine over 5, 25, or 100 sec for the first time) to serial infusions of 50 μg/kg nicotine given over 5, 25 or 100 sec, four days after the last treatment session (**C**; values = mean % acute ± SEM). In **C**, a value of "100" (which represents the locomotor response of saline-pretreated animals given nicotine over 5, 25, or 100 sec for the first time) indicates no change from acute, a negative value indicates a decrease from acute, and a positive value indicates an increase from acute. The x-axis labels in **C** show the speed at which nicotine was infused during the treatment phase as well as on the challenge test day. Also included is a diagram illustrating the serial drug administration protocol (**A**; depicting five individual infusions at each rate, and the inter-infusion interval in each condition) used on each treatment day and on the challenge test day.

situ hybridization of c-*fos* mRNA using an ^{35}S-UTP and -CTP labeled riboprobe complementary to c-*fos* (680-mer, linearized from c-*fos* plasmid donated by Dr. T. Curran, St. Jude Children's Research Hospital, Memphis, Tennessee), and brain sections containing the caudate-putamen were processed for single in situ hybridization of *arc* mRNA (730-mer, linearized from *arc* plasmid donated by Dr. P. Worley, Johns Hopkins School of Medicine, Baltimore, Maryland). Relative mRNA levels were quantified as described in Samaha et al (2004) in the caudate-putamen (divided into dorsal and ventral sectors), the nucleus accumbens (divided into core and shell), the medial prefrontal cortex (divided into Cg1/prelimbic [Prl] and infralimbic [IL] areas), the orbital cortex (divided into ventral and lateral regions), the cingulate cortex, (divided into Cg1 and Cg2 areas), the sensorimotor cortex, the agranular cortex, and the septum.

Double In Situ Hybridization. The caudate-putamen is a heterogeneous structure composed of two major subpopulations of projection neurons. One population preferentially expresses mRNA for preproenkephalin (i.e., Enk+ cells) and forms the striatopallidal pathway, while the other population does not express preproenkephalin mRNA (i.e., Enk- cells) and forms the striatonigral pathway (Gerfen 1992). Using dual in situ hybridization histochemistry we examined the influence of delivery rate on the c-*fos* response to nicotine in Enk+ and Enk- cells in the caudate-putamen. Previous experiments in our laboratory have been conducted to examine the degree to which preproenkephalin mRNA is co-localized with either preprodynorphin or preprotachykinin mRNAs in the caudate-putamen. These co-localization studies demonstrated that the majority (96.3% of 2154 cells) of cells containing the preproenkephalin probe were not co-labeled with either the preprodynorphin or preprotachykinin probes (Uslaner et al 2003b).

Sections containing the caudate putamen were processed for dual in situ hybridization of a ^{35}S-UTP and -CTP labeled riboprobe complementary to c-*fos* mRNA and a digoxigenin-UTP labeled riboprobe complementary to preproenkephalin mRNA (693-mer, linearized from preproenkephalin plasmid courtesy of Dr. J. Douglass, Amgen, Thousand Oaks, California) using a method adapted from Curran and Watson (1995) and described in detail by Ferguson et al (2004). The radioactive riboprobe was generated as described above.

Quantification of Double In Situ. Integrated density measurements of c-*fos* mRNA at several rostrocaudal levels of the caudate-putamen indicated that the effect of infusion rate was greatest in the dorsal caudate-putamen at level –.8 mm relative to

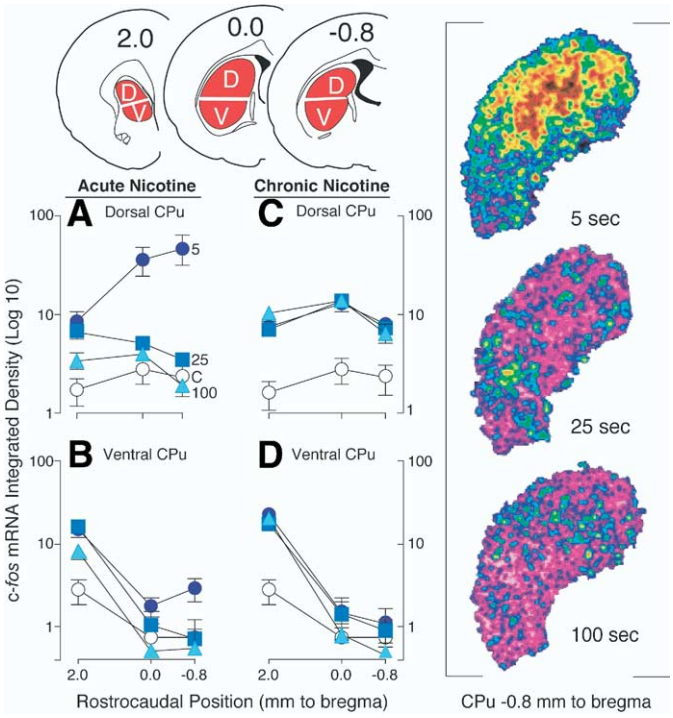

Figure 3. c-*fos* mRNA density in the dorsal and ventral Caudate-Putamen (CPu) following serial infusions of saline or 50 μg/kg nicotine given over 5, 25, or 100 sec in animals previously treated with nicotine or saline (values = mean ± SEM). Also included are representative densitograms illustrating c-*fos* mRNA density in the CPu of saline-pretreated animals given nicotine over 5, 25, or 100 sec for the first time.

bregma (see Figure 3). This region was therefore selected for analysis of double-labeled cells. Total numbers of single- and double-labeled cells were counted in two 250 μm^2 grids in each hemisphere using a Leica microscope (Leitz DMR, Wetzler, Germany) at 200X total magnification. Data from the two hemispheres of each animal were subsequently combined for statistical analysis. Digoxigenin-labeled cells (containing preproenkephalin mRNA) appeared as a purple precipitate under brightfield conditions, and ^{35}S-labeled cells (containing c-*fos* mRNA) were seen as densely packed silver grain clusters under darkfield conditions.

Statistical Analyses

Locomotor Sensitization (Experiments 1-2a). On Day 1, the influence of infusion rate on the acute locomotor response to nicotine was assessed using a one-way analysis of variance (ANOVA) followed by Tukey's Multiple Comparison Test. The influence of infusion rate on the development of locomotor sensitization was assessed using planned paired *t*-tests comparing locomotor counts on Day 1 and Day 6 for each group. Sensitization is indicated by a significant increase in locomotor activity from Day 1 to Day 6.

IEG Expression (Experiment 2b). In brain regions where IEG expression was assessed at one rostrocaudal level (e.g., the mPFC), in the analysis of c-*fos* mRNA density in Enk- vs. Enk+ striatal cells, as well as in the analysis of the change in the IEG response to nicotine in drug-experienced relative to drug-naive animals, group differences were assessed using a one-way ANOVA followed by Tukey's Multiple Comparison Test. In brain regions where IEG expression was assessed at several rostrocaudal levels (e.g., the CPu and the Nacc), group differences were first assessed using an overall two-way ANOVA (condition X rostrocaudal position). If either the main effect or interaction term was significant, two-way ANOVAs comparing the experimental groups to control and to each other were performed.

Results

Experiment 1: Locomotor Sensitization to Single Daily Infusions of Nicotine

When injected for the first time (Day 1), 25 μg/kg nicotine increased locomotor activity significantly above saline only when it was infused over 5 or 25 sec (data not shown, one-way ANOVA followed by Tukey's Multiple Comparison Test; $F = 3.05$, all ps $< .05$), although there was no significant effect of infusion rate. There was no difference in the locomotor response to 25 μg/kg nicotine between Day 1 and Day 6, at any infusion rate tested, indicating that none of the animals developed behavioral sensitization at this dose (data not shown).

On Day 1, 50 μg/kg nicotine produced a greater locomotor response than saline, at all infusion rates tested (Figure 1D, $F = 12.11$, all ps $< .05$), and there was no effect of infusion rate (see Figure 1). It can be seen from visual inspection of Figure 1A-C that the locomotor response to intravenous nicotine was very brief, at all infusion rates tested, and that group differences occurred in the peak locomotor response to the drug over treatment days. Figure 1D summarizes these data, illustrating peak drug response (first 2 min). The peak locomotor response to nicotine increased significantly from Day 1 to Day 6 in animals given infusions over 5 sec (Figure 1D, paired *t*-test; $t = -3.91$, $p = .005$), indicating that these animals sensitized. However, there was no significant difference between Day 1 and Day 6 in animals given infusions over 25–100 sec (ps = .08–.81), indicating that these animals did not sensitize. In addition, there was a significant effect of infusion rate: the magnitude of the increase in the peak locomotor response to nicotine from Day 1 to Day 6 was greater following infusions given over 5 sec than over 100 sec (two-way ANOVA; condition X day interaction, $F = 10.94$, $p < .01$). No other comparisons were significant.

Experiment 2a: Locomotor Sensitization Following Multiple Daily Infusions of Nicotine

In all nicotine-treated groups, the locomotor response to nicotine returned to baseline within 12 min of drug treatment. Therefore, all statistical analyses were performed on the locomotor response averaged over the first 12 min following treatment. On Day 1, serial infusions of 25 μg/kg nicotine increased locomotor activity relative to saline, at all infusion rates tested (data not shown, one-way ANOVA followed by Tukey's Multiple Comparison Test; $F = 8.5$, all ps $< .05$), but there was no effect of infusion rate. There was no difference in the locomotor response to 25 μg/kg nicotine between Day 1 and Day 6, at any infusion rate tested, indicating that none of the groups sensitized at this dose (data not shown). Therefore, data from animals treated with serial infusions of 25 μg/kg nicotine were not analyzed further, and all subsequent data are from animals given 50 μg/kg nicotine.

On Day 1, 50 μg/kg nicotine increased locomotor activity more than saline, at all infusion rates tested (Figure 2B; $F = 37$, all ps $< .001$), and there was no effect of infusion rate (see Figure 2B). On Day 6, only animals infused with nicotine over 5–25 sec (but not 100 sec) showed a significant increase in locomotor activity relative to Day 1 (Figure 2B; paired *t*-tests, Day 1 vs. Day 6; 5 sec, $t = 12.85$, $p < .0001$, 25 sec, $t = 2.99$, $p < .02$, 100 sec, $t = 1.04$, $p = .32$), indicating that only these groups sensitized. In addition, the magnitude of the increase in locomotor activity from Day 1 to Day 6 was greater when nicotine was infused over 5 sec than over 25 sec (two-way ANOVA, condition X day interaction, $F = 14.66$, $p < .002$), or 100 sec ($F = 19.43$, $p < .0003$).

Figure 2C shows the effect of treatment condition on the locomotor response to a challenge test given four days after the last treatment session, depicted as percent of control (i.e., that elicited by acute nicotine treatment; see Figure 2 legend). In control animals previously treated with saline, there was no effect of infusion rate on the locomotor response to nicotine (data not shown, one-way ANOVA, $F = 1.4$, $p = .94$), consistent with what was seen on Day 1 in nicotine-treated animals (see Figure 2B). Sensitization is indicated by a response significantly greater than control, and on the challenge test day only animals previously treated with nicotine infusions over 5 sec showed significant sensitization (unpaired *t*-test, $t = 4.1$, $p < .007$). Animals that were previously treated with nicotine infusions over 25 sec showed a trend towards sensitization ($t = 2.08$, $p = .052$), and animals in the 100 sec group did not differ from control ($t = 1.21$, $p = .24$). In addition, locomotor activity on the challenge test day was significantly greater in the 5 sec group relative to the 100 sec group (one-way ANOVA followed by Tukey's Multiple Comparison Test, $F = 4.5$, post-hoc, $p < .05$).

Experiment 2b: IEG Expression in Response to Serial Daily Infusions of Nicotine

Caudate-Putamen (CPu). Figure 3 illustrates the effect of serial infusions of saline or 50 μg/kg nicotine given over 5, 25, or 100 sec on c-*fos* mRNA expression in the dorsal and ventral CPu

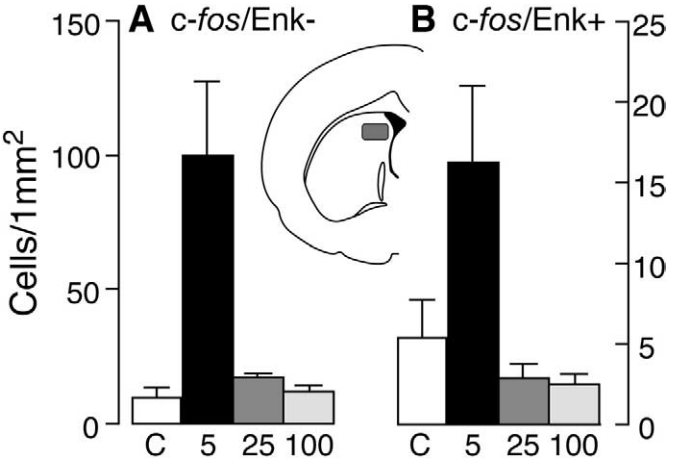

Figure 4. The effect of infusion rate on nicotine-induced c-*fos* mRNA expression in Enk- versus Enk+ cells within the dorsal CPu of animals pretreated with saline and thus receiving nicotine for the first time (values = mean ± SEM). CPu, caudate-putamen.

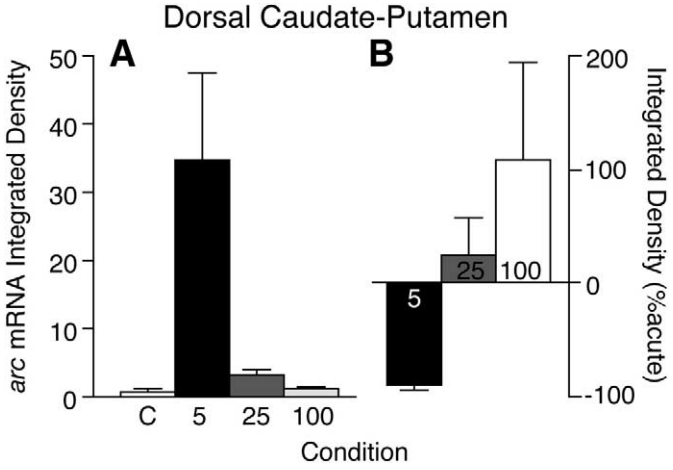

Figure 5. The effect of infusion rate on nicotine-induced *arc* mRNA expression in the dorsal CPu (level –.8) of animals previously treated with saline and receiving nicotine for the first time (**A**; values = mean ± SEM), and nicotine-induced *arc* mRNA expression in the dorsal CPu of animals previously treated with nicotine illustrated as percent change from animals given nicotine for the first time (**B** acute; values = mean % acute ± SEM). In this analysis, a value of "0" (which represents the *arc* response of saline-pretreated animals given nicotine over 5, 25, or 100 sec for the first time) indicates no change from acute, a negative value indicates a decrease from acute, and a positive value indicates an increase from acute. CPu, caudate-putamen.

of animals previously treated with nicotine or saline. In animals given nicotine for the first time (acute nicotine), all drug treatments induced greater c-*fos* mRNA expression than saline in the dorsal and ventral CPu (Figure 3A,B; 2-way ANOVAs, condition X rostrocaudal position, Fs = 3.56–5.91, ps < .05), and there was an effect of infusion rate in both CPu subdivisions (Figure 3A, level 2.0: 5 > 100, levels .0 and –.8: 5 > 25-100; Figure 3B, level –.8: 5 > 25–100, one-way ANOVAs, followed by Tukey's Multiple Comparisons Tests, Fs = 3.30–8.05, all ps < .05). In addition, there were qualitative differences in the rostrocaudal distribution of c-*fos* expression in the dorsal CPu as a function of rate of nicotine delivery (Figure 3A). Following rapid (5 sec) nicotine infusions, c-*fos* expression was greatest in the caudal CPu, and following slower (100 sec) infusions c-*fos* levels were greatest in the rostral CPu (Figure 3A; 5 sec: level –.8 > level 2.0, 100 sec: level .0 > level −.8, one-way repeated-measures ANOVAs, followed by Tukey's Multiple Comparisons Tests, Fs = 6.2–6.9, all ps < .05). This indicates that nicotine must engage different neural networks depending on the rate it is delivered. In the ventral CPu, c-*fos* expression was greatest in rostral sections, at all infusion rates tested (Figure 3B; level 2.0 > other levels, in all groups, all ps < .05).

The effect of infusion rate on c-*fos* expression was greatest at level –.8 of the dorsal CPu, therefore, we analyzed c-*fos* expression in Enk- versus Enk+ cells (Figure 4A,B), as well as *arc* expression (Figure 5A) at this level of the dorsal CPu. In this portion of the CPu nicotine increased c-*fos* mRNA expression in Enk- and Enk+ cells only when given over 5 sec, and 5-sec infusions led to a greater increase in c-*fos* expression in both cell populations relative to infusions given over 25–100 sec (one-way ANOVAs, followed by Tukey's Multiple Comparisons Tests, Fs = 5.98–9.32, all ps < .05; Figure 4). In the dorsal CPu, nicotine increased *arc* mRNA expression above saline only when administered over 5 sec (Figure 5A; F = 7.2, ps < .01), and infusions over 5 sec led to the greatest increase in *arc* expression relative to all other conditions (ps < .01).

In animals previously given repeated injections of nicotine, a nicotine challenge increased c-*fos* levels in the dorsal and ventral CPu above control (Figure 3C,D; 2-way ANOVAs, condition X rostrocaudal position, Fs = 3.51–74.04, ps < .05), but there was no longer any effect of infusion rate. In these animals there was also no effect of infusion rate on nicotine-induced c-*fos* expression in Enk- and Enk+ cells (one-way ANOVAs, ps= .08–.28; data not shown). It is clear from visual comparison of Figure 3A,C that chronic exposure to nicotine altered the ability of a nicotine challenge to induce c-*fos* expression in the dorsal CPu, and that this effect varied with infusion rate. This is depicted in Figure 6A, which shows nicotine-induced c-*fos* expression in the dorsal CPu (level –.8) of animals previously exposed to nicotine, illustrated as percent change from that seen in animals given nicotine for the first time (acute nicotine; see Figure 6 legend). Previous

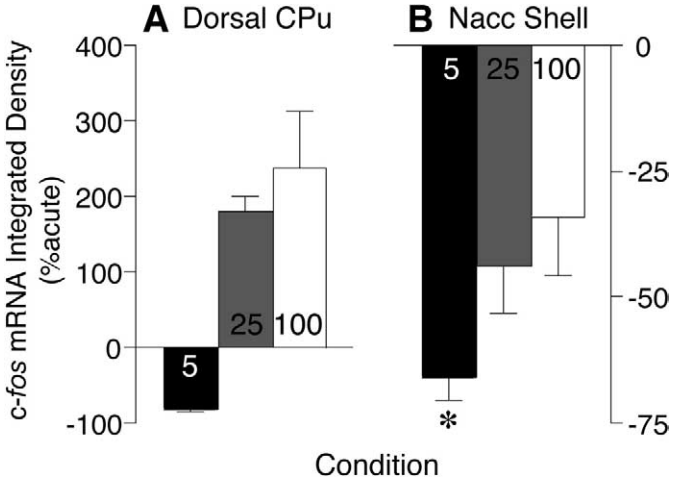

Figure 6. Nicotine-induced c-*fos* expression in the dorsal CPu (Panel **A**; level –.8) and Nacc shell (**B**; level 2.0) of animals previously treated with nicotine illustrated as percent change from animals given nicotine for the first time (acute; values = mean % acute ± SEM). In this analysis, a value of "0" (which represents the c-*fos* response of saline-pretreated animals given nicotine over 5, 25, or 100 sec for the first time) indicates no change from acute, a negative value indicates a decrease from acute, and a positive value indicates an increase from acute. CPu, caudate-putamen.

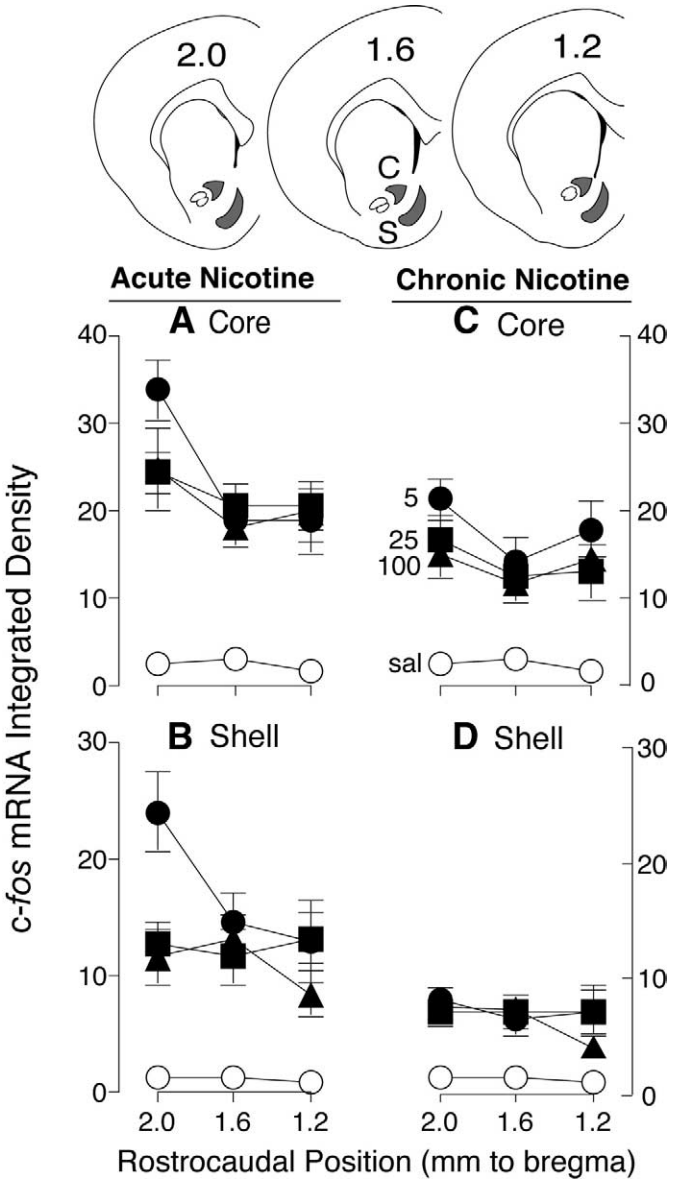

Figure 7. c-*fos* mRNA expression in the Nucleus accumbens (Nacc) core and shell following serial infusions of 50 μg/kg nicotine given over 5, 25, or 100 sec in animals previously treated with saline or nicotine (values = mean ± SEM).

exposure to rapid infusions (5 sec) of nicotine reduced the c-*fos* response to a drug challenge, while previous exposure to slower infusions (25–100 sec) enhanced this response (one-sample *t*-tests, *t*s = −32.15–4.67, *p*s < .02). As depicted in Figure 5B, previous exposure to rapid infusions (5 sec) of nicotine also significantly reduced the *arc* response to a drug challenge (one-sample *t*-tests, $t = -23.08$, $p < .0001$), while previous exposure to slower infusions (25–100 sec) had no effect (*t*s = .72–1.28, *p*s = .24–.49).

Nucleus Accumbens (Nacc). In animals given nicotine for the first time (acute), nicotine increased c-*fos* mRNA expression in the Nacc core and shell, at all infusion rates tested (Figure 7A,B; 2-way ANOVAs, 5 sec vs. sal, condition X rostrocaudal position, *F*s = 4.18–19.83, *p*s < .03, 25–100 sec vs. sal, main effects of condition, *F*s =39.34–145.60, *p*s < .0001). There was also a significant effect of infusion rate, but only in the rostral Nacc shell (i.e., level 2.0 relative to bregma, 5 > 25–100; one-way ANOVA, followed by Tukey's Multiple Comparisons Test, $F = 6.83$, all *p*s < .05). The effect of infusion rate on c-*fos* expression in the core of the Nacc was not statistically significant (Figure 7A).

In all animals previously treated with nicotine, a nicotine challenge increased c-*fos* expression above control in the core and shell (Figure 7C,D; main effect of condition, *F*s = 22.6–60.26, *p*s < .0002), and there was no effect of infusion rate. In addition, it is clear from visual comparison of Figure 7B,D that chronic nicotine reduced the ability of a drug challenge to elicit c-*fos* expression in the rostral shell (level 2.0), and that this effect varied with infusion rate. This is illustrated in Figure 6B, which shows nicotine-induced c-*fos* expression in the Nacc shell (level 2.0) of nicotine-treated animals, depicted as percent change from that elicited by acute nicotine. Although chronic drug treatment reduced the c-*fos* response to a nicotine challenge at all infusion rates tested (one-sample *t*-tests, *t*s = 7.83–8.91, *p*s < .0001), this effect was greatest following treatment with rapid infusions (5 > 100, one-way ANOVA, followed by Tukey's Multiple Comparisons Test, $F = 3.44$, all *p*s < .05). Repeated drug treatment did not significantly change c-*fos* responsiveness in the Nacc core, at any infusion rate tested.

Medial Prefrontal Cortex (mPFC). In animals given nicotine for the first time, all drug treatments increased c-*fos* expression in the Cg1/Prl and IL cortices above saline (Figure 8A,B; one-way

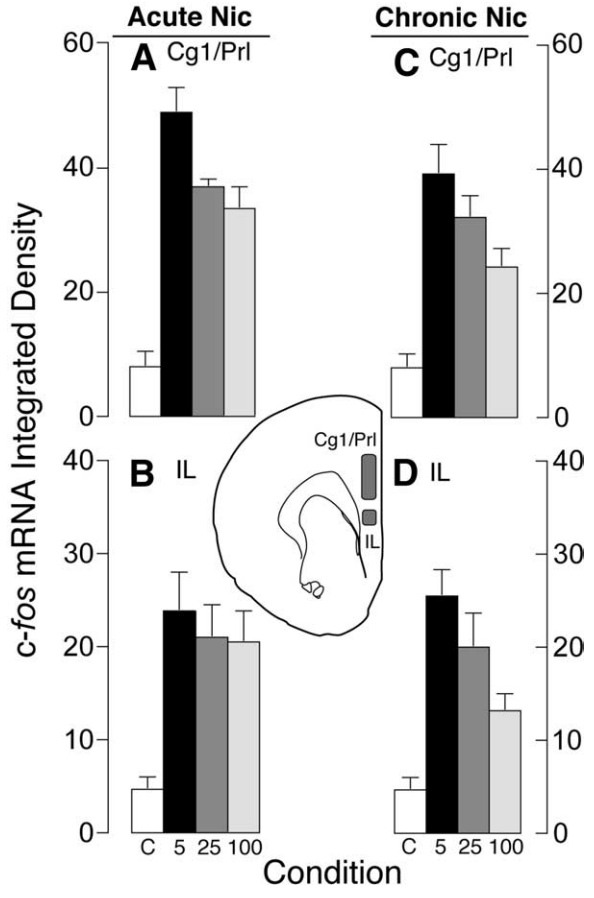

Figure 8. c-*fos* mRNA expression in the Cg1/prelimbic (Cg1/Prl) and infralimbic (IL) aspects of the medial prefrontal cortex following serial infusions of 50 μg/kg nicotine given over 5, 25, or 100 sec in animals previously treated with saline or nicotine (values = mean ± SEM).

ANOVAs followed by Tukey's Multiple Comparison tests, Fs = 7.7–34, all ps < .05), and there was a significant effect of infusion rate in the Cg1/Prl cortex (Figure 8A, 5 > 25 = 100 sec, ps < .05), but not in the IL cortex (Figure 8B).

In all animals previously treated with nicotine, a drug challenge increased c-*fos* expression above saline in the Cg1/Prl and IL cortices (Figure 8C,D; Fs = 12–15, all ps < .05; the 100 sec group did not differ from control in the IL cortex). There was also a significant effect of infusion rate in both cortical subdivisions (5 > 100 in both regions, ps < .05).

The density of c-*fos* mRNA was also quantified in the septum, and the orbitofrontal, cingulate, insular, and somatosensory cortices. In all these regions, nicotine increased c-*fos* expression above saline at all infusion rates tested, and in both drug-experienced and drug-naive animals (data not shown, ps < .05). There was, however, no effect of infusion rate on c-*fos* expression in any of these brain regions. Thus, the influence of infusion rate on the ability of nicotine to increase c-*fos* expression appears to be specific to mesocorticolimbic regions.

Discussion

The idea that the more rapidly drugs of abuse reach the brain the greater their propensity to produce addiction is a central dogma in addiction research (Gossop et al 1992, 1994; Hatsukami and Fischman 1996; Winger et al 1992). This is thought to be one reason, for example, why nicotine inhaled in tobacco smoke is particularly addictive, whereas nicotine taken orally or transdermally is less likely to lead to addiction (Henningfield and Keenan 1993; Hughes 1989; West et al 2000). Rapidly administered drugs are thought to be more addictive because this enhances their euphorigenic properties (de Wit et al 1993; Hatsukami and Fischman 1996). Indeed, self-reports of euphoria are increased when a number of addicting drugs are delivered rapidly (Abreu et al 2001; de Wit et al 1992,1993; Fischman and Schuster 1984; Kollins et al 1998; Mumford et al 1995). However, there may be no necessary causal relationship between the euphorigenic properties of drugs and their addictive potential (Robinson and Berridge 1993). Moreover, although nicotine produces pleasant effects (Kalman 2002), it is unlikely that nicotine produces a euphoric state of such vividness and intensity as to compel smokers to smoke up to dozens of cigarettes a day.

Another reason rapidly administered drugs are thought to be particularly addictive is because this is more reinforcing. This idea comes from a limited number of studies showing that increasing the rate of intravenous administration (between 5–240 sec) enhances the ability of cocaine (Balster and Schuster 1973; Kato et al 1987; Panlilio et al 1998) and nicotine (Wakasa et al 1995) to support drug self-administration behavior in monkeys. However, we have recently found that varying the rate of amphetamine or cocaine delivery between 5–100 sec has no effect on the acquisition of self-administration, on frequency of responding under two fixed ratio schedules of reinforcement, on "breakpoint" achieved on a progressive ratio schedule of reinforcement, or on the reinstatement of drug-seeking following extinction of operant responding in rats (Crombag et al 2003). These findings are consistent with an earlier study using rats (Pickens et al 1969), and suggest that the reinforcing efficacy of psychostimulant drugs may not be affected by small variations in drug delivery rate (i.e., over 5–100 sec).

The ability of drugs to produce adaptations in the mesocorticolimbic system, adaptations that are manifest behaviorally as psychomotor and/or incentive sensitization, is also thought to contribute to addiction (Robinson and Berridge 1993, 2000). Furthermore, the initial phases of many forms of drug experience-dependent plasticity involve drug-induced activation of IEGs (Hyman and Malenka 2001; Nestler 2001). We hypothesized, therefore, that the rapid delivery of nicotine may more readily produce behavioral sensitization and alter nicotine's ability to induce IEG expression in the mesocorticolimbic system. Repeated exposure to rapid (5 vs. 25–100 sec) infusions of nicotine enhanced the development of behavioral sensitization. These results are consistent with our previous observations that rapid cocaine infusions increase susceptibility to behavioral sensitization (Samaha et al 2002, 2004). The current findings are also noteworthy when one considers that nicotine inhaled in tobacco smoke reaches the brain in 10 to 20 sec (Le Houezec 2003). Of course, sensitization has been observed following intraperitoneal or subcutaneous nicotine injections (Benwell and Balfour 1992; Clarke and Kumar 1983; Domino 2001; Hakan and Ksir 1988; Ksir et al 1985; Miller et al 2001; Stolerman et al 1973), both of which would result in the slower uptake of nicotine relative to an IV infusion. It is likely that higher doses of nicotine, and/or a greater number of drug treatments would render sensitization less dependent on rapid delivery. Nonetheless, our results suggest that the rate of nicotine delivery influences the susceptibility to behavioral sensitization, and by inference, the neurobiological adaptations responsible for sensitization.

To begin to explore these neurobiological processes, we examined the cells and circuits engaged by nicotine as a function of infusion rate using c-*fos* and *arc* mRNAs. In agreement with previous results (Kiba and Jayaraman 1994; Mathieu-Kia et al 1998; Matta et al 1993; Pich et al 1997; Ren and Sagar 1992; Salminen et al 1996), acute nicotine administration increased IEG expression in a number of brain regions, at all infusion rates tested. However, the unique finding reported here is that the c-*fos* and *arc* response to nicotine was enhanced by rapid delivery, and that this effect was specific to mesocorticolimbic regions (i.e., the CPu, the Nacc shell, and the mPFC). This is consistent with previous findings showing that rapid cocaine delivery potentiates its ability to increase glucose utilization (Porrino 1993) and to evoke IEG expression (Samaha et al 2004) in mesocorticolimbic structures. Interestingly, rapid (5 sec) infusions enhanced the IEG response to nicotine in the Nacc shell, but had no effect in the Nacc core. These findings are reminiscent of reports that acute nicotine treatment increases extracellular DA levels preferentially in the Nacc shell (Pontieri et al 1995). While the functional significance of changes in gene regulation in the shell remains to be determined, the shell is often cited as mediating the primary reinforcing and stimulatory effects of drugs of abuse, while the core appears to play a preferential role in mediating behavioral responses to conditioned reinforcers (Balfour 2002; Di Chiara 1998; Ito et al 2004; Pontieri et al 1995; Sellings and Clarke 2003).

Previous work has shown that conditions that facilitate the development of sensitization (e.g., pairing psychostimulant administration with a novel environment) also facilitate drug-induced gene expression in Enk+ cells within the CPu (Badiani et al 1999; Uslaner et al 2003a). To our knowledge, the phenotype of striatal cells engaged by nicotine has never been examined before. We found that when administered over 25–100 sec nicotine did not significantly increase c-*fos* expression in Enk+ cells, but when given over 5 sec nicotine did increase c-*fos* expression in these cells, suggesting that the neural circuits engaged by nicotine are modulated by the temporal pattern of drug delivery. The idea that nicotine engages different neural networks as a function of the rate it is delivered is also supported by the observation that the rostrocaudal pattern of IEG expres-

sion in the dorsal CPu is very different when nicotine is given over 5 sec than when it is given over 25 or 100 sec.

We do not know how rate of nicotine delivery alters its ability to induce IEG expression, but different actions may be involved in different brain structures. For example, the primary mechanism by which nicotine induces IEG expression in the striatum may be by activating nicotinic receptors on DA neurons in the midbrain, thereby increasing striatal DA release (Schilstrom et al 2000). In the mPFC nicotine may induce IEG expression more directly via activation of local nicotine receptors (Schilstrom et al 2000). It is possible, therefore, that increasing the rate of delivery may potentiate nicotine's stimulatory effects on DA release, which would lead to greater IEG expression in the striatum, and greater sensitization. In the mPFC, rapidly administered nicotine may enhance c-*fos* induction by altering the temporal dynamics of local nicotine receptor occupancy, which in turn may influence activation of the intracellular signaling pathways that lead to IEG expression, and modulate sensitization.

Additionally, a number of other neurotransmitter systems may be involved. For example, glutamate receptor antagonists block nicotine-induced behavioral sensitization (Shim et al 2002; Shoaib et al 1997; Shoaib and Stolerman 1992), and Fos expression (Kiba and Jayaraman 1994; Schilstrom et al 2000). Furthermore, presynaptic nicotinic acetylcholine receptors control the release of many neurotransmitters, including norepinephrine, serotonin, GABA, glutamate, and acetylcholine (MacDermott et al 1999; Wonnacott 1997), all of which may participate in the effect of infusion rate on nicotine-induced sensitization and IEG expression. Finally, the rate of drug delivery may modulate the response to nicotine by altering nicotinic receptor number and/or function (Hicks et al 2000; Lester and Dani 1995; Mansvelder et al 2002; Mansvelder and McGehee 2000; Pidoplichko et al 1997; Wooltorton et al 2003).

Of course, another possible explanation is that with slower infusions the total dose of nicotine achieved is decreased by metabolism and less drug reaches the brain. However, varying infusion rate had no effect on the acute locomotor response to nicotine, which is dose-dependent (Ksir 1994; Ksir et al 1985). In addition, the influence of infusion rate on the IEG response to nicotine was specific to mesocorticolimbic regions, and there was no effect of infusion rate on c-*fos* expression in the septum, the orbitofrontal, cingulate, agranular, and sensorimotor cortices. However, to reliably exclude any effects of achieved dose, it will be important to directly measure brain levels of nicotine under these conditions.

In addition to modulating the neurobiological impact of an acute nicotine exposure, the rate of delivery also influenced the development of adaptations in the ability of nicotine to induce IEG expression as a consequence of repeated drug treatment. In the CPu, repeated exposure to rapidly administered nicotine decreased the c-*fos* and *arc* response to a subsequent nicotine challenge, while treatment with slower infusions either enhanced (in the case of c-*fos*) or did not affect (in the case of *arc*) nicotine-induced gene expression. Although the functional significance of these changes is not known, one interesting possibility is that the time course of nicotine-induced adaptations in gene regulation may vary as a function of delivery rate.

In closing, our findings demonstrate that rapidly administered nicotine increases susceptibility to sensitization, and more readily engages the mesocorticolimbic system. We propose that rapidly administered drugs may increase the likelihood of addiction not simply because they are more euphorigenic or are more reinforcing, but because they lead to a preferential induction of forms of drug experience-dependent plasticity that promote compulsive drug-taking and relapse. The implication, therefore, is that by rapidly delivering nicotine to its sites of action, the nicotine delivery system itself (i.e., cigarettes) may contribute to making cigarette smoking one of the hardest addictions to break.

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