Review

The Reinforcing Effects of Nicotine in Humans and Nonhuman Primates: A Review of Intravenous Self-Administration Evidence and Future Directions for Research

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Abstract

Introduction: Cigarette smoking is largely driven by the reinforcing properties of nicotine. Intravenous (IV) self-administration procedures are the gold standard for investigating the reinforcing effects of psychoactive drugs. The goal of this review was to examine the results of published investigations of the reinforcing effects of nicotine measured using IV self-administration procedures in humans and nonhuman primates.

Results: The body of literature using nonhuman primate subjects indicates nicotine functions as a positive reinforcer when available for self-administration via IV catheters. However, it can also be difficult to establish IV nicotine self-administration in nonhuman primates and sometimes supplemental strategies have been required (e.g., priming injections or food deprivation) before subjects acquire the behavior. Although the body of literature using human subjects is limited, the evidence indicates nicotine functions as a reinforcer via the IV route of administration in adult cigarette smokers. Rates of nicotine self-injection can be variable across subjects and responding is sometimes inconsistent across sessions in both humans and nonhuman primates.

Conclusions: The Family Smoking Prevention and Tobacco Control Act, enacted in 2009, gave the Food and Drug Administration regulatory authority over the manufacture, marketing, and distribution of tobacco products. Research examining the threshold reinforcing doses for initiation and maintenance of nicotine self-administration, comparisons of the reinforcing effects of nicotine in adolescent versus adult subjects, investigations of gender differences in the reinforcing effects of nicotine, and studies of the abuse liability of non-nicotine tobacco product constituents and their ability to alter the reinforcing effects of nicotine will inform potential tobacco regulatory actions.

Introduction

The Centers for Disease Control and Prevention (CDC) reported that 18% of adults in the United States were cigarette smokers in 2012. Among adult smokers, the CDC estimates nearly 90% began smoking before the age of 18. Compared to people who have never smoked, life expectancy for cigarette smokers is decreased by at least one decade and more than 480,000 Americans die from smoking related illnesses every year, making tobacco use the largest preventable cause of death and disease in the United States. From 2009–2012, at least $133 billion was spent on direct medical care related to cigarette smoking, and lost productivity costing $156 billion was attributed to cigarette smoking. With the passage of the Family Smoking Prevention and Tobacco Control Act in 2009, the U.S. Food and Drug Administration (FDA) was given authorization...
to regulate the manufacture, marketing, and distribution of tobacco products. The FDA’s Center for Tobacco Products has three public health goals: prevent youth initiation of tobacco use, lower the harm and addictiveness of tobacco products, and encourage cessation of tobacco use.

Cigarette smoking appears to be largely driven by the reinforcing properties of nicotine. According to Henningfield and Goldberg, the behavioral and physiological effects of smoking tobacco were described as early as the 1920s and nicotine was identified as the principal pharmacologic component in tobacco by biomedical researchers Armstrong-Jones in 1927 and Lewin in 1931. The addictive properties of nicotine were acknowledged in internal tobacco industry communications in the 1950s, with cigarettes being described as an ideal nicotine delivery device. The scientific consensus that nicotine plays a primary role in initiating and maintaining smoking behavior was first described in the 1964 Surgeon General’s report and confirmed in subsequent reports. The average daily intake of nicotine via cigarette smoke is difficult to estimate given differences in behavior amongst smokers and the variable nicotine content across cigarette brands. Cigarettes may contain 1–14 mg of nicotine, with 0.01–1.5 mg of nicotine being absorbed from smoking a single cigarette.

There is generally excellent correspondence between drugs that are abused by humans and those that are self-administered by laboratory animals. Unlike other behavioral paradigms that may be used to indirectly examine the reinforcing effects of a drug (e.g., conditioned place preference), self-administration procedures provide direct measures of behavior (e.g., lever pressing) that is maintained by the reinforcing effects of a drug. Accordingly, the self-administration paradigm is the gold standard for assessing the reinforcing effects of psychoactive compounds. In addition, the role of other variables (e.g., dose, schedule of reinforcement, volume and duration of injections, and prior drug and training experience) in mediating the reinforcing effects of drugs can be systematically investigated using self-administration procedures.

Many early attempts to demonstrate the reinforcing effects of nicotine using intravenous (IV) self-administration procedures in rodents were unsuccessful. Several factors may have contributed to these initial negative findings including the age and strain of rat, injection speeds, limited duration of catheter patency, drug and training histories, and the reinforcement schedule used. Nonetheless, more recent studies have reported reliable and robust nicotine self-administration in rats. In addition to contributing to the overall understanding of the reinforcing effects of nicotine, results from rodent studies are exceptionally useful for informing investigations in nonhuman primates and humans. Moreover, since nicotine is not scheduled according to the Controlled Substances Act and is freely available for human consumption via tobacco products and other nicotine delivery devices, the reinforcing effects of IV self-administered nicotine have also been evaluated in humans. A review of all data related to the reinforcing effects of nicotine is beyond the scope of this review, which focuses on investigations of the reinforcing effects of IV nicotine in humans and nonhuman primates. Indeed, a December 2014 PubMed search using the relatively specific term “nicotine self-administration” returned over 1,400 related references. Thus, one aim of this review is to describe the history and progress of research focused on assessing the reinforcing effects of IV nicotine in nonhuman primates and humans. A second aim is to review specific research areas related to the reinforcing effects of tobacco product constituents that will provide empirical data and inform the FDA in its mission to promote public health through the regulation of tobacco products.

### Results

#### IV Self-Administration of Nicotine in Nonhuman Primates

Table 1 summarizes the methods and results from studies of the reinforcing effects of IV nicotine in nonhuman primates. One of the first attempts to establish nicotine self-administration in a nonhuman primate species was carried out by Deneau and Inoki in 1967. Initially, nicotine was available for self-injection in seven rhesus monkeys (unspecified gender and age). When spontaneous nicotine self-administration did not occur, a response-independent injection (i.e., priming injection) was delivered every hour. When nicotine self-administration was still not established following two months of hourly priming injections, the automatic injections ceased and the nicotine dose was increased and spontaneous nicotine self-administration occurred in two subjects. Following additional automatic nicotine injections over 2–10 days, nicotine self-administration was initiated in the remaining five subjects (i.e., priming injections were required). The average daily total intake of nicotine self-administered was variable and ranged from 0.7 to 1.7 mg/kg. The dose of nicotine per injection was then increased each month until self-administration ceased. Of the six monkeys tested in this fashion, one stopped at 0.05 mg/kg/injection, two stopped at 0.1 mg/kg/injection, one stopped at 0.5 mg/kg/injection, one stopped at 1.0 mg/kg/injection, and one continued to self-administer at the highest dose tested (2.0 mg/kg/injection). At the 2.0 mg/kg/injection dose, the subject self-administered an average daily dose of 9.6 mg/kg. Unfortunately, details about the number of injections, and pattern and rate of responding across sessions and subjects were not provided. Thus, we cannot determine if the results from these studies satisfy any currently acceptable criteria for determining if a drug functions as a reinforcer (i.e., dose-dependent changes in responding, comparison to responding for vehicle injections). In addition, many methodological details were not reported (e.g., injection volume, duration of injection, time-out periods). Nonetheless, the authors concluded that under the conditions employed in their study, nicotine was self-administered intravenously in rhesus monkeys without a prior history of operant conditioning using drug or food reinforcement.

The importance of a subject’s experimental history and the duration of access to the test compound (i.e., session duration) was evident in another early nicotine self-administration study by Yanagita (see also Yanagita et al.). Given 24-hr access to nicotine injections, six out of six rhesus monkeys (unspecified gender and age) described as “self-administration experienced” by the authors, self-injected at least one dose of nicotine relative to saline injections. Only four out of seven experimentally naive subjects did so when nicotine was substituted for saline. The average total daily intake of nicotine ranged from 0.2–2.0 mg/kg. Importantly, nicotine injections consistently maintained responding above vehicle levels. When access was limited to 4 hr, however, nicotine was not self-administered in “experienced” monkeys when it was substituted for a psychostimulant (levetamine) that was already maintaining self-administration. It has been well-established that experimental history and session duration can influence self-administration behavior.

The first to report nicotine maintained responding above vehicle levels in an old world nonhuman primate species. Another key factor related to pattern of drug self-administration is the timeout period following a self-injection. In adult male baboons (Papio anubis and Papio hamadryas) previously trained to lever press for food or other drugs as reinforcers, Griffiths et al. reported that nicotine failed to maintain responding above vehicle levels during 24-hr sessions of nicotine availability with concurrent...
### Table 1. Nicotine IV Self-Administration (SA) in Nonhuman Primates

<table>
<thead>
<tr>
<th>Year</th>
<th>Authors</th>
<th>Species</th>
<th>Subject history</th>
<th>Dose range (mg/kg/injection)</th>
<th>Session duration</th>
<th>Schedule</th>
<th>Time out</th>
<th>Methodological strategies</th>
<th>Doses self-administered (mg/kg/injection)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1967</td>
<td>Deneau and Inoki</td>
<td>rhesus</td>
<td>naive (n = 7)</td>
<td>0.01–2.0</td>
<td>24 hr</td>
<td>FR 1</td>
<td>unspecified</td>
<td>priming injections when spontaneous SA didn’t occur</td>
<td>0.025–2.0</td>
</tr>
<tr>
<td>1977</td>
<td>Yanagita</td>
<td>rhesus</td>
<td>drug SA (n = 4); drug SA (n = 6); naive (n = 7)</td>
<td>0.0025–0.6; 0.02–0.2; 0.02–0.2</td>
<td>unspecified</td>
<td>FR 1</td>
<td>nicotine substituted for lefetamine; nicotine substituted for saline; nicotine substituted for saline</td>
<td>0.02–0.2</td>
<td></td>
</tr>
<tr>
<td>1979</td>
<td>Griffiths et al.</td>
<td>baboon</td>
<td>drug and food SA (n = 3); naive (n = 1)</td>
<td>0.001–3.2; 0.03</td>
<td>24 hr</td>
<td>FR 160</td>
<td>3 hr</td>
<td>nicotine substituted for cocaine; priming injections of nicotine during initial training; training required 2–9 months using unspecified methods</td>
<td>0.015–0.09</td>
</tr>
<tr>
<td>1981</td>
<td>Goldberg et al.</td>
<td>squirrel</td>
<td>cocaine SA (n = 3); naive (n = 1)</td>
<td>0.0015–0.27</td>
<td>3 hr</td>
<td>subject dependent</td>
<td>unspecified</td>
<td>nicotine substituted for cocaine, saline, and nicotine baselines; nicotine substituted for cocaine; FI duration varied as a manipulation</td>
<td>0.01–0.1</td>
</tr>
<tr>
<td>1982</td>
<td>Dougherty et al.</td>
<td>rhesus</td>
<td>not described (n = 4)</td>
<td>0.003–0.56</td>
<td>2 hr or 10 injections</td>
<td>FI</td>
<td>1 min</td>
<td>nicotine substituted for cocaine, saline, and nicotine baselines; nicotine substituted for cocaine; FI duration varied as a manipulation</td>
<td>0.003–0.05</td>
</tr>
<tr>
<td>1983</td>
<td>Goldberg and Spealman</td>
<td>squirrel</td>
<td>cocaine SA (n = 2); food SA (n = 2); naive (n = 1)</td>
<td>0.0003–0.056</td>
<td>2 hr or 10 injections</td>
<td>FI</td>
<td>1 min</td>
<td>nicotine substituted for cocaine, saline, and nicotine baselines; nicotine substituted for cocaine; FI duration varied as a manipulation</td>
<td>0.0003–0.05</td>
</tr>
<tr>
<td>1983</td>
<td>Spealman and Goldberg</td>
<td>squirrel</td>
<td>drug and food SA (n = 4); naive (n = 2); drug SA (n = 3)</td>
<td>0.003–0.1</td>
<td>2 hr or 10–12 injections</td>
<td>second order</td>
<td>1–3 min</td>
<td>nicotine substituted for cocaine, saline, and nicotine baselines; nicotine substituted for cocaine; FI duration varied as a manipulation</td>
<td>0.003–0.05</td>
</tr>
<tr>
<td>1983</td>
<td>Ator and Griffiths</td>
<td>baboon</td>
<td>drug and food SA (n = 3); naive (n = 1)</td>
<td>0.01–0.32</td>
<td>2 hr or 50 injections</td>
<td>FR 2</td>
<td>15 s</td>
<td>nicotine substituted for cocaine, saline, and nicotine baselines; nicotine substituted for cocaine; FI duration varied as a manipulation</td>
<td>0.01–0.01</td>
</tr>
<tr>
<td>1984</td>
<td>Slifer</td>
<td>rhesus</td>
<td>not described (n = 3)</td>
<td>0.0001–0.1</td>
<td>1 hr</td>
<td>FR 1</td>
<td>unspecified</td>
<td>nicotine SA w/ and w/o concurrent food pellet SA</td>
<td>0.03–0.1</td>
</tr>
<tr>
<td>1985</td>
<td>Slifer and Balster</td>
<td>rhesus</td>
<td>drug SA (n = 1); naive (n = 2)</td>
<td>0.0001–0.1</td>
<td>1 hr</td>
<td>FR 1</td>
<td>unspecified</td>
<td>nicotine SA w/ and w/o concurrent food pellet SA</td>
<td>0.01–0.1</td>
</tr>
<tr>
<td>1987</td>
<td>De La Garza and Johanson</td>
<td>rhesus</td>
<td>drug SA (n = 1); naive (n = 2)</td>
<td>0.01–0.3</td>
<td>1 hr</td>
<td>FR 10</td>
<td>unspecified</td>
<td>nicotine substituted for cocaine</td>
<td>0.01</td>
</tr>
<tr>
<td>1994</td>
<td>Sannerud et al.</td>
<td>squirrel</td>
<td>cocaine SA (n = 4)</td>
<td>0.01–0.1</td>
<td>1 hr or 12 injections</td>
<td>FR 30</td>
<td>5 min</td>
<td>nicotine substituted for cocaine</td>
<td>0.008–0.06</td>
</tr>
<tr>
<td>1995</td>
<td>Wakasa et al.</td>
<td>rhesus</td>
<td>drug SA (n = 8)</td>
<td>0.03</td>
<td>24 hr</td>
<td>FR 5</td>
<td>15 min</td>
<td>nicotine substituted for saline; infusion speed varied as a manipulation</td>
<td>0.03</td>
</tr>
</tbody>
</table>
Table 1. Continued

<table>
<thead>
<tr>
<th>Year</th>
<th>Authors</th>
<th>Species</th>
<th>Subject history</th>
<th>Dose range (mg/kg/injection)</th>
<th>Session duration</th>
<th>Schedule</th>
<th>Time out</th>
<th>Methodological strategies</th>
<th>Doses self-administered (mg/kg/injection)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>Le Foll et al.</td>
<td>squirrel</td>
<td>naive ($n = 5$)</td>
<td>0.003–0.03(^a) 1 hr</td>
<td>FR 10</td>
<td>1 min</td>
<td>distinct visual stimulus paired w/ nicotine injections</td>
<td>0.003–0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.003–0.06(^a) 6 hr or 0.5 hr w/o a response</td>
<td>PR</td>
<td>unspecified</td>
<td>nicotine substituted for cocaine or saline; also evaluated nicotine + cocaine mixtures</td>
<td>0.003–0.06</td>
<td></td>
</tr>
<tr>
<td>2009</td>
<td>Freeman and Woolverton</td>
<td>rhesus</td>
<td>drug SA ($n = 4$); no drug SA ($n = 1$)</td>
<td>0.012–0.05(^a) 1.5–13 hr(^b)</td>
<td>PR</td>
<td>30 min</td>
<td>nicotine substituted for cocaine; also evaluated nicotine + cocaine mixtures</td>
<td>0.012–0.05(^a)</td>
<td></td>
</tr>
<tr>
<td>2011</td>
<td>Gould et al.</td>
<td>rhesus</td>
<td>cocaine and food SA and/or drug discrimination ($n = 8$)</td>
<td>0.01–0.1(^a) 16 hr or 2 hr w/o an injections</td>
<td>PR</td>
<td>10 min</td>
<td>nicotine substituted for cocaine; also evaluated effects of varenicline on cocaine SA</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>2011</td>
<td>Mello et al.</td>
<td>rhesus</td>
<td>not described ($n = 5$)</td>
<td>0.00032–0.1(^a) 1 hr or 20 injections</td>
<td>second order</td>
<td>10 s</td>
<td>nicotine substituted for cocaine; also evaluated nicotine + cocaine mixtures</td>
<td>0.0032–0.01</td>
<td></td>
</tr>
<tr>
<td>2011</td>
<td>Justinova et al.</td>
<td>squirrel</td>
<td>nicotine SA ($n = 4$)</td>
<td>0.03 1 hr</td>
<td>FR 10</td>
<td>1 min</td>
<td>distinct visual stimulus paired w/ nicotine injections; evaluating 2-arachidonoylglycerol</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>2011</td>
<td>Mascia et al.</td>
<td>squirrel</td>
<td>nicotine SA ($n = 3$)</td>
<td>0.03 1 hr</td>
<td>FR 10</td>
<td>1 min</td>
<td>nicotine substituted for cocaine; also evaluated nicotine + cocaine mixtures</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>2012</td>
<td>Panlilio et al.</td>
<td>squirrel</td>
<td>“nicotine experienced” ($n = 4$)</td>
<td>0.03 1 hr</td>
<td>FR 10</td>
<td>1 min</td>
<td>nicotine substituted for cocaine; also evaluated nicotine + cocaine mixtures</td>
<td>0.03</td>
<td></td>
</tr>
</tbody>
</table>

\(FI = \text{fixed-interval schedule of reinforcement}; FR = \text{fixed-ratio schedule of reinforcement}; \) PR = progressive-ratio schedule of reinforcement.

\(^a\) Nicotine dose range expressed as the free base.

\(^b\) Basis (salt or free base) of nicotine dose range not described.

\(^c\) Nicotine dose range expressed as the salt.

\(^d\) Dose range included if nicotine self-administration occurred in at least one subject.

\(^e\) The same data, with additional details, was presented in Yanagita et al.\(^33\)

\(^f\) PR value not specified.

\(^g\) 1-1,2-diphenyl-1-dimethylaminoethan HCL (SPA; lefetamine).

\(^h\) The doses self-administered were determined by a general visual inspection of the data presented or statements made by the authors, but statistical analysis to support such conclusions were not presented in the referenced citation.

\(^i\) During initial training, the first response after 5 min elapsed produced an injection; if no response occurred within 7 min then an automating (priming) injection occurred.

\(^j\) Data for 2 subjects were presented; one subject worked on a second order schedule where completion of an FR 4 produced a 0.5-s tone and the completion of a FR 4 after a FI (1 min) produced the tone and an injection; the second subject worked on a tandem FR/FI schedule where the first response after an injection initiated a 16-s interval and the first response after the interval produced an injection.

\(^k\) Nicotine SA in 3 out of 4 subjects.

\(^l\) Parameters (maximum number of injections, duration of FI, duration of timeout) varied across subjects.

\(^m\) Patterns of responding for nicotine injections differed across baseline conditions (cocaine, saline, nicotine) and an inverted u-shaped dose-effect curve was observed in the first 5 days of nicotine SA but became flatter as responding stabilized.

\(^n\) Sessions ended after 20 injections or two consecutive 30-min trials without completion of the response requirement; each injection was followed by 30-min timeout.

\(^o\) Nicotine self-administered in only one of five subjects and responding was not dose-dependent.
unlimited food access. A 3-hr timeout period followed each nicotine injection, limiting the number of injections to 8 per 24-hr session. In a follow-up study, Ator and Griffiths used shortened sessions (2hr) and brief timeout periods (15 or 60 s) and reported that nicotine was self-administered significantly above saline levels in three out of three subjects. Other methodological differences between the two studies included the response requirement and substitution procedures. In the Griffiths et al. study, a fixed ratio (FR) schedule of reinforcement was used and nicotine was substituted for cocaine, but nicotine was not self-administered above vehicle levels. In the Ator and Griffiths study, results from two experiments were presented. In one, an FR schedule (15-s timeout) was used to examine nicotine self-administration during 2-hr sessions. Nicotine self-administration was variable in this experiment, with patterns of responding for nicotine injections differing across baseline conditions (cocaine, saline, and nicotine baselines) and with higher rates of nicotine self-administration occurring during the first 5 days of nicotine access compared to the last 5 days. In the second experiment, a fixed interval (FI) schedule (60-s timeout) was used and nicotine was substituted for a cocaine baseline. Responding was less variable compared to the first experiment and the number of nicotine injections was higher than that of saline injections. The highest total mean intake of nicotine per 2-hr session ranged between 1.0–2.8 mg/kg across subjects. Thus, in addition to differences in session duration and timeout periods, the schedule of reinforcement also differed between the two studies. The FI schedule produced less variable response rates for nicotine injections across time when compared to the FR schedule. The authors also noted that even when responding for nicotine injections was higher than for vehicle injections, the overall response rates for nicotine injections were relatively low when compared to rates maintained by other drugs (e.g., cocaine). Importantly, the Ator and Griffiths study was the first to demonstrate an inverted-U-shaped dose-response curve for nicotine self-administration in non-human primates.

Goldberg et al. first demonstrated that high rates of behavior could be maintained by nicotine self-injections in adult male squirrel monkeys (Saimiri sciureus) using an unlimited food access condition. Goldberg and colleagues used a second-order schedule of reinforcement under which completion of an FR requirement following a FI of time resulted in changes in the color of a stimulus light; completion of an additional FR requirement then initiated a nicotine injection that was paired with another change in the color of the stimulus light. Response rates during experimental sessions were characterized by the authors as “consistently high” and averaged between 0.81 and 1.58 responses per second. When saline injections were substituted for nicotine injections, response rates fell to very “low levels” (actual number not given). Since experimental sessions ended after the 12th timeout period (or 90 min elapsed), the maximum number of nicotine injections that could be earned was 12. Using an average squirrel monkey body weight of 0.8 kg, the maximum total nicotine intake that could have been self-administered would have been approximately 0.36 mg/kg. Pretreatment with mecamylamine, a nicotinic antagonist, resulted in lowered rates of responding for nicotine injections. Subsequent studies reported full nicotine dose-response curves in adult male squirrel monkeys. In all three of these squirrel monkey studies, automatic (i.e., priming) injections of nicotine were used to help establish nicotine self-administration in some monkeys. During the initial training, the first response after 5 min produced an injection of nicotine. However, if no response occurred within 7 min, an automatic injection of nicotine occurred. A comparison of responding under both an FI and the second-order schedule of reinforcement demonstrated that patterns of responding maintained by nicotine were qualitatively similar to those maintained by cocaine or morphine injections using these schedules of reinforcement.

Overall rates of responding for nicotine self-injections were higher under the second-order schedule compared to the FI schedule and the authors attributed this difference to the visual stimuli paired with each nicotine injection. Specifically, under the FI schedule, one stimulus light indicated injections could be earned and was extinguished during timeout periods. Under the second order schedule, however, injections were associated with a brief change in the color of the stimulus light. Thus, these studies were the first to demonstrate a meaningful role for the presentation of unique visual stimuli paired with nicotine self-injection and the selective sensitivity of the reinforcing effects of nicotine to blockade by a nicotinic antagonist (mecamylamine) in a new world monkey species.

Around the same time the Goldberg and Spealman research group was examining the reinforcing effects of nicotine in squirrel monkeys, others were using rhesus monkeys. Compared to squirrel monkeys, similar rates of responding for nicotine injections were reported for rhesus monkeys (gender and age unspecified) when comparable schedules of reinforcement were used. The highest total nicotine intake self-administered during 3-hr sessions, according to the data for the two subjects shown, appears to have ranged from approximately 2–13 mg/kg under a “slow” substitution regimen. Dougherty and colleagues compared responding during two dose-change procedures. In the “rapid” procedure, the nicotine dose was changed daily (progressive increase and then a progressive decrease over daily sessions), while in the “slow” procedure the nicotine dose was changed every 5–10 sessions. The data presented for two subjects indicate higher response rates, more injections, and a greater amount of nicotine was self-administered using the “slow” procedure. Thus, Dougherty et al. was the first to demonstrate the importance of the substitution procedures chosen for assessing the reinforcing effects of nicotine. This has become an area of interest as a possible strategy for lowering the abuse liability and harm from tobacco products (see below “Future Directions”).

Other studies in adult male rhesus monkeys used FR schedules of reinforcement and sought to determine if concurrent responding for food or food deprivation would alter nicotine self-administration. When food pellets were available under an FI schedule on one lever, and injections of nicotine were available under an FR schedule on a different lever, higher rates of responding for nicotine were found when compared to a “no food” condition. While nicotine maintained self-administration under both food deprivation and satiation conditions in three adult rhesus monkeys, nicotine intake was higher under food deprivation conditions in one of three subjects. A second subject showed a similar trend but the shift was not statistically significant. These results suggest that food deprivation may affect the potency and effectiveness of nicotine as a reinforcer.

Another variable that may impact self-administration behavior is the duration of nicotine infusions. Durations of injections and rates of nicotine infusion for studies that provided such information, or included enough information to estimate infusion rates, are shown in Table 2. The impact of rate of infusion (0.0003–0.0052 mg/s) on nicotine self-administration in male and female rhesus monkeys, and the corresponding time-course of nicotine plasma levels, was examined in a study by Wakasa et al. They reported that as the time to infuse a fixed nicotine dose (0.03 mg/kg/
Table 2. Infusion Rates in Nicotine IV Self-Administration (SA) Studies

| Year | Authors | Species | Infusion duration (s) | Infusion rate (ml/s) | Infusion rate (mg/s) | Injection volume | Dose range | SA?
|------|---------|---------|----------------------|---------------------|---------------------|-----------------|-----------|-----
| 1977 | Yanagita | rhesus  | 46                   | 1/23                | 0.00043–0.114      | 0.25mg/kg       | 0.025–0.64mg/kg/injection | no  |
| 1979 | Griffiths | baboon  | 46                   | 1/23                | 0.00043–0.114      | 0.25mg/kg       | 0.025–0.64mg/kg/injection | yes |
| 1982 | Goldberg | squirrel | 0.2                 | 2/20.2              | 0.012–2.20          | 0.25mg/kg       | 0.030–0.56mg/kg/injection | yes |
| 1982 | Spealman | squirrel | 0.2                 | 2/20.2              | 0.012–2.20          | 0.25mg/kg       | 0.030–0.56mg/kg/injection | yes |
| 1983 | Ator     | baboon  | 5                   | 1/5                 | 0.050–1.60          | 0.25mg/kg       | 0.030–0.56mg/kg/injection | yes |
| 1983 | Henningfield | human | 10                  | 1/10                | 0.075–0.30          | 0.25mg/kg       | 0.030–0.56mg/kg/injection | yes |
| 1983 | Henningfield | baboon | 9.2               | 1/9.2               | 0.082–0.33          | 0.25mg/kg       | 0.030–0.56mg/kg/injection | yes |
| 1983 | Slifer   | rhesus  | 10                  | 1/10                | 0.0008–0.08         | 0.25mg/kg       | 0.030–0.56mg/kg/injection | yes |
| 1985 | Slifer   | rhesus  | 10                  | 1/10                | 0.0008–0.08         | 0.25mg/kg       | 0.030–0.56mg/kg/injection | yes |
| 1987 | De La Garza & Johanson | rhesus | 10                  | 1/10                | 0.0064–0.20         | 0.25mg/kg       | 0.030–0.56mg/kg/injection | yes |
| 1994 | Sannerud | squirrel | 0.2                 | 2/0.2               | 0.040–0.40          | 0.25mg/kg       | 0.030–0.56mg/kg/injection | yes |
| 1995 | Wakasa   | rhesus  | 33                  | 1/23                | 0.0052              | 0.25mg/kg       | 0.030–0.56mg/kg/injection | yes |
| 2003 | Rose     | human   | 2                   | not described       | 0.02–0.075          | not described   | individualized  | yes |
| 2004 | Harvey   | human   | 10                  | 1/10                | 0.075–0.30          | 1ml             | 0.75–3.0mg/kg/injection  | yes |
| 2007 | Le Foll  | squirrel | 0.2                 | 2/0.2               | 0.040–0.40          | 0.25mg/kg       | 0.030–0.56mg/kg/injection | yes |
| 2008 | Sofuoglu | human   | 30                  | 5/30                | 0.0033–0.23         | 0.25mg/kg       | 0.030–0.56mg/kg/injection | yes |
| 2009 | Freeman  | rhesus  | 10                  | not described       | 0.012–0.050         | not described   | 0.030–0.56mg/kg/injection | yes |
| 2010 | Rose     | human   | 180–510             | not described       | 0.6–1.90            | not described   | individualized  | yes |
| 2011 | Gould    | rhesus  | 10                  | 1.5/10              | 0.0080–0.09         | 1.5ml           | 0.01–0.1mg/kg/injection  | no  |
| 2011 | Mello    | rhesus  | 1                   | 0.1/1               | 0.0026–0.80         | 0.1ml           | 0.0032–0.1mg/kg/injection | yes |
| 2011 | Justinova| squirrel | 0.2                | 0.2/0.2             | 0.12                | 0.2ml           | 0.03mg/kg/injection       | yes |
| 2011 | Mascia   | squirrel | 0.2                | 0.2/0.2             | 0.12                | 0.2ml           | 0.03mg/kg/injection       | yes |
| 2012 | Panlilio | squirrel | 0.2                | 0.2/0.2             | 0.12                | 0.2ml           | 0.03mg/kg/injection       | yes |

aNicotine dose range expressed as the free base.
bBasis (salt or free base) of nicotine dose range not described.
cNicotine dose range expressed as the salt.
dThe same data, with additional details, were presented in Yanagita et al.47

Calculation(s) based on other information provided by the authors and/or using estimated mean body weights (e.g., 6–10kg for rhesus monkeys, 20–25kg for baboons, 0.8kg for squirrel monkeys, 75–80kg for humans).

Griffiths et al.31 describe the injection duration as occurring “within a 2-min period” (p.168).

5-mL drug solution followed by 5-mL vehicle.

Doses of nicotine that were administered were individualized and calculated to be equal to a puff on the usual brand of cigarette during a baseline session.

The duration and volume of injection for 0.01mg/kg/injections of nicotine; other doses were achieved by varying the duration and volume of injections.

Set to be equal to each individual’s rate of intake during cigarette smoking.

Injection) was increased (slower infusion speed), the average rates of nicotine self-administration decreased. In addition, plasma concentrations of nicotine were positively correlated with infusion speeds in mg/s: the faster the injections, the higher the plasma nicotine levels. These data suggest that the rate of infusion may affect responding for injections of nicotine. In contrast, the Griffiths et al.31 study in adult male baboons reported that nicotine (0.001–3.2mg/kg/injection) did not maintain responding above vehicle levels using infusion rates up to approximately 100-fold faster than that of Wakasa et al.47 (i.e., infusion rates of 0.00017–0.53mg/s as calculated using 20kg as the mean baboon body weight). A subsequent study in adult male baboons46 reported clear inverted U-shaped dose-response curves for nicotine self-administration (0.01–0.1mg/kg/injection) using infusion rates of approximately 0.05–1.6mg/s. The FR-160 schedule of reinforcement used by Griffiths et al.,31 however, was a much higher response requirement than the FR-5 schedule used by Wakasa et al.47 and the FR-2 schedule used by Ator and Griffiths.46 Thus, the lack of a demonstration of nicotine self-administration in the Griffiths et al.31 study may have been due to the relatively high FR requirement. Indeed, a more recent study reported nicotine failed to maintain responding above saline levels when substituted for cocaine under a progressive ratio schedule of reinforcement in adult male rhesus monkeys.48 Specifically, the final ratio completed (i.e., breakpoint) was less than 100 for nicotine injections, compared to more than 900 for cocaine.44 A similar study reported that nicotine maintained self-administration above vehicle levels in only one of five adult male rhesus monkeys under a progressive ratio schedule.49 The maximal infusion rates of IV nicotine in the studies of Freeman and Woolverton49 and Gould et al.44 were much faster than that used in the study of Wakasa et al.47 Thus, though one study indicated that lower infusion rates decreased nicotine self-administration,47 the body of data shown

In 2007, adult naive male squirrel monkeys with no prior operant behavioral training, no food deprivation or concurrent food self-administration, with no automatic (i.e., priming) injections of nicotine, but with the use of associated auditory and visual stimuli, engaged in high rates of nicotine self-administration. Since rodent studies had suggested that the reinforcing effects of nicotine were, in part, due to its enhancing the reinforcing effectiveness of environmental stimuli, a colored stimulus light was paired with nicotine injections and a different colored light was paired with completion of the FR requirement on a second “inactive” lever. Responding on each lever was compared to ascertain if nicotine injections were affected by the presentation of the different stimuli. Subjects responded significantly more on the lever associated with the nicotine-paired visual stimulus than on the lever paired with only a visual stimulus. Thus, in the absence of behavioral and drug histories and without employing facilitating conditions (e.g., priming injections, food deprivation), nicotine was an effective positive reinforcer in adult male squirrel monkeys. The ability of IV nicotine injections to reinforce behavior in experimentally naive subjects was shown to be similar to that of cocaine. The highest nicotine intake appears to have been approximately 0.35 mg/kg during 1 hr sessions. Later studies conducted by the same research group reported an approximate average nicotine intake of 1.5 mg/kg during 1-hr sessions in adult male squirrel monkeys. At this point, however, it is unknown whether IV nicotine injections are reinforcing in the absence of changes in associated visual and auditory stimuli in experimentally naive nonhuman primate subjects.

Additional studies in nonhuman primates have focused on assessing the preclinical efficacy of various pharmacological interventions to decrease or stop nicotine self-administration (e.g., medications development). For example, after demonstrating that nicotine, cocaine, and combinations of nicotine and cocaine maintained responding above vehicle levels in rhesus monkeys, the effects of chronic treatment with buspirone and varenicline on self-administration were examined. The effects of clofibrate, WY14643 and methOEA, and sertraline on nicotine self-administration have also been examined. As discussed below (“Future Directions”), the reinforcing effects of non-nicotine tobacco product constituents (e.g., anatabine) are also being investigated in squirrel monkeys.

Thus, the body of literature in nonhuman primates confirms that nicotine functions as a positive reinforcer when available for self-administration via IV catheters (Table 1). Nicotine is self-administered in multiple nonhuman primate species, in both experienced and naive subjects, and across multiple experimental conditions (e.g., various schedules of reinforcement, timeout periods, and duration of infusions). Nicotine self-administration, however, can also be difficult to establish in nonhuman primates, sometimes requiring supplemental strategies (e.g., priming injections or food deprivation) before subjects acquire the behavior. Rates of nicotine self-injection can also be variable across subjects and inconsistent across sessions within a study. It has been suggested that non-nicotine tobacco product constituents may enhance the reinforcing effects of nicotine, or have reinforcing effects on their own that contribute to maintaining nicotine self-administration behavior via smoking. Thus, it is possible that nicotine self-administration would be more easily acquired and/or consistent in the presence of other tobacco product constituents. Indeed, examinations of non-nicotine tobacco product constituents are essential areas of study for future assessments of the reinforcing effects of nicotine (see below “Future Directions”).

### IV Self-Administration of Nicotine in Humans

While the reinforcing effects of nicotine have been designated as the primary factors in the maintenance of cigarette smoking, relatively little research in humans has specifically examined the reinforcing effects of nicotine outside of the context of smoking. Table 2 summarizes the results from investigations of IV nicotine self-administration studies in adult cigarette smokers. In 1983, Henningfield and colleagues examined IV nicotine self-administration in adult male smokers. The total nicotine intake was described for each subject for one dose (1.5 mg/injection) during one session and ranged from 0.018–0.027 mg/kg. While neither the number of injections nor the rate of responding across nicotine doses and saline were described, the authors concluded that nicotine was, indeed, self-administered and that when compared to saline, nicotine response patterns were less variable and didn’t decrease across sessions while saline injections did. In a second study, both nicotine self-administration and avoidance of nicotine administration were evaluated in male smokers. The authors reported that nicotine maintained higher rates of responding when compared to saline in “some” subjects (i.e., positive reinforcing effects maintained self-administration behavior) but in other subjects, lower rates of responding were observed when compared to saline (i.e., aversive effects of nicotine suppressed self-administration behavior). Interestingly, subjects reported adverse effects of nicotine (e.g., nausea, fear, coughing, and pain at injection site) regardless of whether nicotine was maintaining or suppressing self-administration behavior. In a subsequent experiment, subjects that did not self-administer nicotine during initial sessions participated in sessions where they could respond to avoid nicotine injections and/or respond to self-inject nicotine. Programmed nicotine injections occurred at specific intervals, but responding on one lever prior to the injection turned off a stimulus light and canceled the next injection (avoidance). Responding on another lever produced a nicotine injection (self-administration). All three subjects that were tested responded to avoid nearly all of the nicotine injections and never responded to self-inject nicotine. Henningfield and his colleagues were the first to demonstrate nicotine self-administration in humans using an IV route of administration, but also that some smokers would respond to avoid nicotine injections.

Rose and colleagues investigated the effects of pretreatment with mecamylamine on IV nicotine self-administration in male and female smokers. The doses per injection were individualized so that they were comparable to the per-puff nicotine doses that subjects obtained when freely smoking their usual cigarette brand during baseline sessions. The study did not include a vehicle control condition for comparison of response rates to nicotine self-administration rates. When no mecamylamine was given, a mean of 3.4 mg (SD = 3.52) nicotine was self-administered. When mecamylamine was given, a mean of 4.3 mg (SD = 3.17) nicotine was self-administered. The authors concluded that nicotinic receptors played a role in the reinforcing effects of nicotine.

Harvey and colleagues used a vehicle control condition in their investigations of IV nicotine self-administration in male smokers and clearly demonstrated nicotine-maintained responding above vehicle
levels. Under a concurrent FR schedule of reinforcement, responding on one lever resulted in a nicotine injection and responding on a different lever resulted in a saline injection. The number of injections was significantly greater for nicotine than for saline, though there was a dose-dependent decrease in the number of nicotine injections at the highest dose tested. In addition, the effect of different FR values (10–1600) and timeout length (1–20 min) on lever responding were evaluated in some subjects. At the four upper FR values (200, 400, 800, and 1600), nicotine maintained responding above saline levels. No significant changes in response rate were associated with the changes in timeout length. Harvey et al. were the first to demonstrate a clear inverted-U dose-response curve for IV nicotine self-administration and responding under a high FR requirement in cigarette smokers.

IV nicotine self-administration was also compared to a vehicle control condition in smokers in a study conducted by Sofuoglu and colleagues. They were interested in doses of nicotine that resembled those expected to be delivered in a typical cigarette puff. During experimental sessions, sample injections from each of two syringes (saline and nicotine) were given, and subjects were then allowed to choose between the two syringes (six choice opportunities/session). The two highest doses of nicotine tested (0.4 and 0.7 mg/injection) were chosen more than saline. Preference for IV nicotine was compared to IV saline and to puffs of denicotinized cigarette smoke and sham puffs by Rose and colleagues in smokers. A baseline smoking session was used to identify the average nicotine dose smoked for each participant, followed by two training sessions in which participants were trained to discriminate between IV nicotine or saline, and then between puffs of denicotinized smoke and sham puffs. Lastly, four experimental sessions occurred in which choice behavior was characterized during choice components of the sessions that followed satiation components. The four possible satiation conditions (one per session) were: denicotinized cigarette puffs + IV nicotine injections; denicotinized cigarette puffs + IV saline injections; sham puffs + IV nicotine injections; and sham puffs + IV saline injections. Satiation components consisted of programmed administration of puffs and injections based on each subject’s unlimited smoking during baseline sessions. During experimental sessions, the first component was a satiation component, followed by a choice component, then a second satiation component, a second choice component, a third satiation component, and then finally a third choice component. The first two choice components provided a choice between only two alternatives: IV nicotine versus IV saline or cigarette puffs versus sham puffs. The third choice component provided all four alternatives. During choice components, subjects controlled the number of puffs and injections. Satiation components were 60 min in duration and choice periods were 10 min in duration. When IV nicotine and IV saline injections were available during either of the first two choice periods, participants demonstrated a significant preference for IV nicotine injections. Denicotinized puffs were selected significantly more than sham puffs during the first two choice conditions. When all four alternatives were available, participants had a significant preference for denicotinized cigarette puffs when compared to sham puffs, nicotine injections, and saline injections. Thus, Rose et al. was the first study to compare the reinforcing effects of non-nicotine tobacco components to those of nicotine in humans. While denicotinized cigarette puffs were chosen most often, this may be due to sensory cues associated with smoking behavior (e.g., smell) or other pharmacologically active non-nicotine tobacco product constituents (e.g., acetaldehyde).

Thus, the body of literature examining the reinforcing effects of nicotine in humans is limited. All studies in humans, however, have reported that nicotine maintained self-injection behavior, indicating nicotine functions as a positive reinforcer via the IV route of administration in adult cigarette smokers. Like studies in nonhuman primates, however, the consistency of nicotine self-injection across subjects and sessions can be variable and one study reported that subjects responded to avoid nicotine injections. While the nonhuman primate data indicate nicotine is self-administered across various experimental conditions (e.g., various schedules of reinforcement and timeout periods), the conditions under which nicotine is self-injected by humans has been limited to the use of FR schedules and choice procedures, and the use of short timeout periods. Like in nonhuman primates, the infusion duration does not seem to play a crucial role in maintaining nicotine self-injection in humans. While the nonhuman primate data indicate environmental variables (e.g., stimulus lights) that are paired with nicotine injections may play a critical role in maintaining nicotine self-injection, this has not been examined in human subjects. In addition, the reinforcing effects of nicotine are unknown in naive human subjects and only one study has examined the effects of non-nicotine tobacco product constituents in humans. Interestingly, the dose range of nicotine that functioned as a positive reinforcer in humans (0.75–3.0 mg/injection; translates to 0.0094–0.034 mg/kg/injection in an 80 kg subject) is similar to the general dose range found to maintain nicotine self-administration in nonhuman primates (general range of 0.003–0.056 mg/kg/injection). The similarities between humans and nonhuman primates on measures of the reinforcing effects of IV self-administered nicotine suggest that nonhuman primate subjects are an especially useful model for future investigations.

Future Directions

Threshold Reinforcing Doses of IV Nicotine

Reducing nicotine levels in tobacco products has been suggested as a strategy for lowering the abuse liability and harm associated with tobacco products. The effects of both gradual and immediate methods of nicotine dose reduction on nicotine self-administration have been studied in rodents. For example, two schedules of nicotine dose reduction and their effects on maintenance of established self-administration have been compared in rats. A “gradual” reduction approach (nicotine doses were systematically lowered by half approximately every 10 sessions after self-administration was established) was compared with an “immediate” reduction approach (nicotine doses were immediately lowered across sessions after self-administration was established) and the results suggested similar behavioral changes occurred with both methods. Both schedules of nicotine dose reduction produced significant decreases in self-administration behavior, accompanied by some temporary compensatory behavior. Thus, it is possible that lowering nicotine levels in tobacco products may result in decreased exposure to nicotine and other tobacco product constituents, and/or elimination of exposure altogether via an extinction of smoking behavior, in a significant proportion of tobacco users. Indeed, the introduction of denicotinized cigarettes to cigarette smokers in controlled laboratory conditions resulted in decreased smoking behavior.

There are two distinct phases of nicotine self-administration (acquisition and maintenance) that may be used to investigate the role of a threshold reinforcing nicotine dose. Reports in rodents generally indicate that higher unit doses of nicotine are required for...
initiation/acquisition of IV nicotine self-administration relative to doses that maintain self-administration once acquired (for review see Donny et al.\textsuperscript{,29}). That is, there appears to be a lower reinforcement threshold for maintenance of nicotine self-administration in rats trained with nicotine than for the acquisition of nicotine self-administration in rats with no history of nicotine reinforcement. Thus, it is possible that lowering nicotine levels in tobacco products may result in fewer people initiating the behavior of nicotine self-administration (e.g., smoking).

The effects of possible nicotine dose-reduction strategies on nicotine self-administration initiation and maintenance behavior have not been characterized in nonhuman primates. It would be unethical to study the threshold nicotine dose associated with initiation of nicotine self-administration in humans. It would be possible, however, to examine the threshold reinforcing doses required to maintain IV nicotine self-administration, along with the effectiveness of immediate and gradual nicotine reduction on IV nicotine self-administration. The design of these kinds of studies in humans would benefit greatly from data obtained from a nonhuman primate species. In addition, results from rodent and nonhuman primate studies would be especially useful for informing human studies examining the threshold reinforcing doses of nicotine required to maintain smoking behavior in and out of a controlled laboratory setting, and the effects of gradual and immediate reduction of nicotine content in cigarettes on subsequent smoking behavior. Thus, addressing the following experimental questions using human and nonhuman primate subjects may provide useful information for potential regulatory actions regarding nicotine levels in tobacco products:

- What is the threshold reinforcing dose of nicotine for acquisition of IV self-administration in a nonhuman primate?
- What is the threshold reinforcing dose of nicotine for the maintenance of IV self-administration in nonhuman and humans?
- Are immediate or gradual nicotine dose reduction schedules more effective at decreasing IV nicotine self-administration in humans and nonhuman primates?

The IV Reinforcing Effects of Nicotine in Adolescents
Adolescence appears to be a particularly important and susceptible developmental period regarding the initiation of substance abuse and tobacco product use specifically. The initiation of tobacco product use occurs most often during adolescence\textsuperscript{,78} and adult smokers who began smoking during adolescence are significantly more likely to become nicotine dependent than those who did not begin smoking
until adulthood. There is little evidence of initiation of tobacco product use after the age of 24. Though the period of adolescence in rats is relatively short (post-natal days 28–55), some investigators have shown age-related susceptibility to the initiation of nicotine self-administration in rats. More specifically, adolescent rats appear to self-administer a wider range of nicotine doses significantly above vehicle levels compared to adult rats regardless of gender. Additionally, peri-adolescent exposure to nicotine has been shown to result in increased nicotine self-administration in adulthood when compared to rats lacking such exposure. Thus, in rodents, it is possible that exposure to nicotine during adolescence may enhance the subsequent rewarding/reinforcing effects of nicotine when exposure occurs in adulthood. Comparisons of nicotine self-administration between adolescents and adults have not been made in nonhuman primates. A nonhuman primate species that experiences an extended adolescent period (similar to humans) would be the more ideal species for comparisons of the effects of nicotine self-administration in adolescent and adult subjects. Moreover, given the long life span of nonhuman primates, studies on the long-term consequences of adolescent exposure to nicotine can more readily be undertaken using nonhuman primate species. It would be unethical to administer nicotine to adolescent human subjects. Thus, addressing the following experimental questions using nonhuman primate subjects will provide useful information for understanding the role of developmental period in the initiation and continued use of tobacco products into adulthood:

- Are there differences in the rates of acquisition of IV nicotine self-administration in adolescent versus adult subjects?
- Are there differences in response rates, patterns of responding, or total number of injections across IV nicotine doses during ongoing nicotine self-administration in adolescent versus adult subjects?
- Are there differences in threshold reinforcing doses of nicotine during the acquisition and/or maintenance of IV nicotine self-administration in adolescent versus adult subjects?
- Are other outcome measures (e.g., pharmacokinetic outcomes) correlated with behavioral differences in the acquisition and/or maintenance of IV nicotine self-administration in adolescent versus adult subjects?

Non-Nicotine Tobacco Product Constituents

Of the thousands of compounds that can be found in tobacco products, nicotine is the primary alkaloid, accounting for approximately 96%–98% of the total alkaloid content in tobacco. The remaining 2%–4% is comprised of nornicotine, anabasine, anatabine, cotinine, and myosmine. In addition, other tobacco constituents include several aldehyde compounds (e.g., acetaldehyde, formaldehyde) and compounds that may act as monoamine oxidase inhibitors (e.g., famesylacetone, harmine, norharmane). The FDA has established a list of harmful and potentially harmful constituents (HPHCs) that are contained in tobacco products. The HPHC list includes constituents that are banned from foods (applies only to smokeless tobacco products), have been identified as known, probably or possible human carcinogens, produce adverse respiratory or cardiac effects, produce developmental toxicity, or result in abuse liability. The abuse potential of a constituent may be evidenced by at least two of the following measures: central nervous system activity, animal drug discrimination, conditioned place preference, animal self-administration, drug liking, or signs of withdrawal. There are currently 93 tobacco product constituents included on the HPHC list, with four of those compounds designated as having abuse liability (acetaldehyde, anabasine, nicotine, and nornicotine). Acetaldehyde, which is also a metabolite of ethanol (and has been studied in this context), maintains IV self-administration in naive, adult, male rats. One study has examined nornicotine and reported it also maintained responding above vehicle levels in experimentally naive adult male rats. Additionally, the reinforcing effects of nicotine alone have been compared to those of nicotine in combination with a mixture of other tobacco alkaloids (anabasine, nornicotine, anatabine, cotinine and myosmine) in rodents. Adult male rats receiving combinations of nicotine plus an alkaloid mixture responded significantly more when compared to those receiving nicotine alone and to those receiving saline in combination with the alkaloid mixture. Both nicotine groups (nicotine alone and nicotine with the alkaloid mixture) responded significantly more than the saline group and the saline with the alkaloid mixture group. Since the alkaloids were not tested individually, the interpretation of these data is problematic. Cigarette smoke extract (CSE) was recently reported to be more effective in both initiating and maintaining self-administration when compared to nicotine alone in adult male rats. However, the CSE-induced enhancement of the reinforcing effects of nicotine was dependent on the schedule of reinforcement since CSE was comparable to nicotine alone in maintaining responding under a progressive-ratio schedule of reinforcement. In addition, nornicotine (but not anabasine nor anatabine), was self-administered above saline levels by adult male rats when substituted for nicotine. Pre-treatment with each of these three alkaloids dose-dependently decreased responding maintained by a range of nicotine doses, and all three produced a nicotine-like discriminative-stimulus effects in adult male mice. Only one study has evaluated the reinforcing effects of a non-nicotine tobacco product constituent in nonhuman primates. In that study, a range of anabasine doses (0.0032–0.32 mg/kg/injection) was substituted for nicotine (0.01 mg/kg/injection) to examine its abuse potential and its efficacy as a pretreatment for nicotine self-administration in adult rhesus monkeys. Mello et al. reported that anatabine failed to maintain responding above vehicle levels when substituted for nicotine in nicotine-experienced animals. When given as a pretreatment, anabine dose-dependently decreased responding maintained by a range of nicotine self-injections (0.001–0.01 mg/kg/injection). Anabine pretreatment, however, also decreased food-maintained responding. Despite the non-specific decreases in responding maintained by nicotine injections or food presentations, the authors concluded that the data suggest anabine may be useful for the treatment of nicotine addiction. Nonetheless it is notable that the tobacco alkaloids are not always nicotine-like or enhancers of the reinforcing effects of nicotine. There is additional evidence of the abuse liability of non-nicotine tobacco product constituents using other behavioral procedures. Conditioned place preference and drug discrimination procedures in rodents indicate that acetaldehyde and nornicotine have considerable abuse potential (for review see Hoffman et al.). In addition, studies using drug discrimination procedures indicate the stimulus effects produced by anabasine and cotinine dose-dependently substituted for the discriminative-stimulus effects of nicotine in adult male rats trained to discriminate nicotine from saline. Only one previous study has evaluated the nicotine-like discriminative-stimulus effects of non-nicotine tobacco product constituents in nonhuman primates. Nornicotine and cotinine produced nicotine-like...
discriminative-stimulus effects in adult male squirrel monkeys.\textsuperscript{107} In contrast anabasine dose-dependently, but only partially (up to approximately 50%), produced nicotine-like discriminative-stimulus effects.\textsuperscript{107} Overall, given the vast number of constituents found in tobacco products, very little research has examined the abuse potential of tobacco product constituents other than nicotine. Results from rodent models should be used to inform future studies in nonhuman primates. Additionally, it is likely that non-nicotine tobacco product constituents may alter the reinforcing effects of nicotine.

Since very few studies have examined the abuse potential or behavioral effects of IV administered behaviorally active doses of the vast majority of non-nicotine tobacco product constituents, it would be unethical to examine such things in humans until data from rodent and nonhuman primate studies have been reported. Experimental questions about the abuse potential of non-nicotine tobacco product constituents and their ability to alter the IV reinforcing effects of nicotine include:

- Do individual non-nicotine tobacco product constituents have IV reinforcing effects (i.e., are they self-administered) in nonhuman primate subjects?
- Do individual non-nicotine tobacco product constituents alter the reinforcing effects of IV nicotine in nonhuman primate subjects?

**Gender Differences**

There is some evidence of gender differences in the reinforcing effects of nicotine in laboratory animals and human smokers (for reviews see Perkins et al.\textsuperscript{108} and Fattore et al.\textsuperscript{109}). Female rats more quickly acquired nicotine self-administration at lower doses and earned more injections of nicotine on a progressive-ratio schedule compared to male rats.\textsuperscript{110} In addition, increased nicotine self-administration was more pronounced in male adolescent rats than in adolescent female rats (compared to adult males and females), though the effect was more persistent over time in females.\textsuperscript{37,79,83,111} In other studies, female rats had higher rates of responding for presentations of a visual discriminative-stimulus without nicotine injections compared to male rats.\textsuperscript{112,113} While a few nonhuman primate studies have used females as subjects,\textsuperscript{33,47,58} no study has systematically compared females and males on measures of the reinforcing effects of IV nicotine. Similarly, human studies have sometimes included female subjects,\textsuperscript{67,68,114} but a systematic comparison of the reinforcing effects of IV nicotine in male and female subjects has not been done.

Some gender comparisons of smoking behavior have been reported. In female cigarette smokers, changes in the nicotine dose contained in cigarettes had less of an effect on the reinforcing properties of smoking when compared to male smokers,\textsuperscript{115} indicating female smokers may be less sensitive to the reinforcing effects of nicotine in cigarettes. Conversely, female rodents\textsuperscript{110} and female smokers\textsuperscript{106,117} may be more sensitive to non-pharmacological stimuli associated with nicotine administration.

Thus, addressing all of the experimental questions posed throughout the Future Directions section in male and female nonhuman primate and human subjects would provide useful information for understanding the role of gender in the initiation and continued use of tobacco products. More specifically:

- Are there gender differences in the threshold dose of nicotine required to initiate and maintain IV self-administration in adult nonhuman primates?
- Are there gender differences in the threshold dose of nicotine required to maintain IV self-administration in adult nonhuman primates?
- Are there gender differences during the initiation and maintenance of IV nicotine self-administration in adolescent nonhuman primates?
- Are there gender differences in the correlation of other outcome measures (e.g., pharmacokinetic outcomes) with IV nicotine self-administration in adolescent and adult nonhuman primates and humans?
- Are there gender differences in the abuse potential of non-nicotine tobacco product constituents in adult and adolescent nonhuman primates?
- Do individual non-nicotine tobacco product constituents alter the reinforcing effects of nicotine differentially in adolescent and adult male and female nonhuman primates?

**Human Studies of the Reinforcing Effects of Nicotine**

In addition to the specific areas identified above using IV self-administration procedures in humans (i.e., threshold reinforcing dose required to maintain IV nicotine self-administration, gender differences in the maintenance of IV nicotine self-administration, gender differences in the correlation of other outcomes measures with IV nicotine self-administration), there are other potentially useful areas that may be applicable to human subjects including:

- What is the threshold reinforcing dose of IV nicotine that maintains smoking behavior in humans under controlled laboratory settings versus naturalistic conditions?
- Are immediate or gradual IV nicotine dose reduction schedules more effective at decreasing smoking behavior in humans?

As noted above, the effects of behaviorally active doses of the vast majority of non-nicotine tobacco product constituents are not known and these compounds, at behaviorally active doses, should not be examined in humans in the absence of data from animal studies. Also described previously, however, one study compared denicotinized cigarettes to IV nicotine in humans,\textsuperscript{86} allowing the reinforcing effects of a combination of non-nicotine tobacco product components to be examined in humans. Recently, the reinforcing effects of cigarette smoke extract have been examined in rodents\textsuperscript{102} and future studies in humans (and nonhuman primates) may find this approach useful for comparing the reinforcing effects of IV nicotine with cigarette smoke extract.

**Summary**

In summary, studies of IV nicotine self-administration procedures in nonhuman primates and humans are particularly useful for addressing several relevant research questions aimed at providing an empirical basis for informing tobacco product regulation. The body of literature indicates that nicotine functions as a positive reinforcer when available for self-injection in nonhuman primates and human cigarette smokers. Further research using nonhuman primates examining the threshold reinforcing doses of IV nicotine that are required to initiate and maintain self-administration, comparing the reinforcing effects of nicotine in adolescent versus adult subjects and male versus female subjects, investigating the abuse potential of non-nicotine tobacco product constituents, and evaluating the effects that non-nicotine tobacco product constituents may have on the reinforcing effects of nicotine will inform the FDA in its mission to promote public health through the regulation of tobacco products.
Funding
U.S. Food and Drug Administration/Center for Tobacco Products (NCTR Protocol E07537-01).

Declaration of Interests
None declared.

Acknowledgments
The information in these materials is not a formal dissemination of information by the FDA and does not represent agency position or policy.

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