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Nicotine discrimination and self-administration in humans as a function of smoking status

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Abstract Nicotine's discriminative stimulus effects may be critical to understanding reinforcement of tobacco smoking. It is not known whether regular nicotine exposure produces tolerance or sensitivity to these effects. In this study, male and female smokers ($n = 11$) and never-smokers ($n = 10$) were trained to discriminate 20 μg/kg nicotine by nasal spray from placebo (0) on day 1. On day 2, both groups were tested on generalization of this discrimination across intermittent presentations of 0, 3, 6, 12, and 20 μg/kg nicotine in random order. Quantitative and quantal behavioral discrimination tasks, used in previous research, were employed. On day 3, subjects were instructed to self-administer sprays from the 20 μg/kg nicotine versus 0 bottles in a concurrent-choice procedure. All but one subject (female smoker) learned reliably to discriminate 20 μg/kg nicotine from placebo (≥ 80% correct) on day 1. Nicotine-appropriate responding on day 2 was attenuated in smokers versus never-smokers at 20 μg/kg on the quantitative task and at 12 μg/kg on the quantal task, suggesting tolerance. There was no difference in responding at other doses. Smokers also showed attenuated responses on the subjective measure of "head rush", which was associated with discrimination responding in both groups. Nicotine self-administration was significantly greater in smokers versus never-smokers, who self-administered nicotine below chance levels, and was inversely related to discrimination behavior in never-smokers but unrelated in smokers. Women smokers showed less change in nicotine-appropriate responding across generalization doses, reported less confidence in discriminating training doses during acquisition on day 1, and tended to self-administer less nicotine on day 3. These results indicate that smokers may become tolerant to the discriminative stimulus effects of nicotine, perhaps promoting increased use.

Key words Nicotine · Discrimination · Self-administration · Subjective effects · Tolerance · Sex differences · Smoking status · Reinforcement

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Introduction

The discriminative stimulus effects of nicotine are very likely critical to its abuse liability (e.g. Stolerman and Jarvis 1995; Perkins and Stitzer 1997). Until recently, nicotine discrimination in humans had been virtually unexplored. However, it has been demonstrated that dependent tobacco smokers are able to discriminate among low doses of nicotine per se, delivered by nicotine nasal spray (Perkins et al. 1994a), and that an acutely manipulated condition, the specific nicotine dose used to train initial discrimination, can alter subsequent discrimination responding (Perkins et al. 1996a). This latter effect demonstrates that the discriminative stimulus effects of nicotine are not fixed properties of the drug but can be influenced by differences in recent experience, in this case the specific training conditions.

It is also likely that differences in nicotine discrimination occur due to long-term, chronic differences between subjects, such as past history of nicotine exposure from smoking tobacco. Attenuated subjective responses to nicotine per se have been found in dependent smokers compared with never-smokers (e.g., Hughes et al. 1989; Perkins et al. 1994b), suggesting the development of chronic tolerance to these effects of nicotine. Because a drug's discriminative stimulus

effects are thought to be closely related to its subjective effects (Preston 1991), chronic tolerance may also develop to nicotine's discriminative stimulus effects. Development of tolerance is considered a critical component in the onset of dependence to most drugs (Kalant et al. 1971). Although tolerance to the discriminative stimulus effects of drugs has received careful attention in the non-human animal literature (Young 1991), there has been almost no such research on tolerance to these effects of nicotine. Moreover, there has been a nearly complete absence of human studies directly comparing sensitivity to a drug's discriminative stimulus effects as a function of past history of exposure (Preston 1991), although some older research indicated that tolerance may develop to alcohol discrimination (Lansky et al. 1978; Lipscomb and Nathan 1980). If humans become tolerant to the discriminative stimulus effects of nicotine with extended exposure (i.e. with the onset of regular smoking), a reduction in sensitivity to differences in dose could help explain the well-known escalation in intake of nicotine over the first few years of exposure to tobacco products (McNeill 1991).

Alternatively, past history of exposure to a drug may be associated with *better* drug discrimination. Bigelow and Preston (1989) suggest that drugs may have different stimulus properties between abusers and naive subjects and, in fact, drug discrimination research with naive subjects may have little relevance to assessing abuse liability. Therefore, rather than smokers showing reduced ability to discriminate nicotine due to chronic tolerance, smokers may show better nicotine discrimination because of familiarity with nicotine effects. The virtual absence of any direct comparison of discriminative stimulus effects of any drugs between abusing and naive human subjects (Preston 1991) precludes a clear prediction of whether smokers would be better or poorer than never-smokers in discriminating nicotine.

Furthermore, although nicotine per se has been shown to be self-administered by humans under some conditions (e.g. Henningfield et al. 1988; Hughes et al. 1989; Perkins et al. 1996b, c, 1997), whether nicotine discrimination is directly related to self-administration is another question that has received virtually no research attention in either the human or animal literatures. In mice, greater nicotine self-administration is associated with reduced sensitivity to nicotine-induced seizures (Robinson et al. 1996), suggesting that tolerance to aversive effects promotes greater intake. In contrast, greater conditioned place preference to nicotine, considered another measure of reinforcement, is related in mice to greater nicotine-induced locomotion (Schechter et al. 1995). Finally, mechanisms responsible for nicotine self-administration versus discrimination appear to be different, with dopamine playing a critical role in self-administration (Corrigall et al. 1992) but very little role in discrimination (Corrigall and Coen 1994) in rats.

This study examined differences in nicotine discrimination and self-administration between regular smokers and never-smokers administered measured doses of nicotine by nasal spray. Attenuated discrimination in smokers would suggest development of tolerance to discriminative stimulus effects of nicotine, while superior discrimination in smokers would suggest that past experience with nicotine is important in being able to discriminate the drug. In addition, we assessed reinforcement from nicotine in a separate session using a concurrent choice self-administration procedure (Perkins et al. 1996b) in order directly to relate nicotine discrimination with reinforcement, a link often assumed but almost never clearly examined.

Materials and methods

Subjects

Subjects were 11 tobacco smokers (six male and five female) and ten never-smokers (five male and five female) similar in age (mean = 24.0 versus 21.9 years, respectively.). (One additional smoker could not reliably discriminate nicotine during training and was excluded.) Groups were also similar on self-reported alcohol intake (5.1 versus 4.8 drinks/week for smokers versus never-smokers, respectively), ruling out possible cross-tolerance between alcohol and nicotine as a potential explanation for any group differences in nicotine discrimination (e.g. Collins et al. 1988). Smokers smoked a mean (range) of 20.1 (15–33) cigarettes/day, had smoked for 5.7 (2–12) years, and had a mean score of 5.6 (4–9) on the Fagerstrom Test of Nicotine Dependence (FTND, 0–10 scale; Fagerstrom and Schneider 1989), typical of FTND values found for smokers in cessation studies and higher than those found in the general population of smokers from the US and other countries (Fagerstrom et al. 1996). All subjects were examined by physician to rule out current or past medical or psychiatric problems, and urine drug screens were obtained to exclude subjects with substance abuse problems (amphetamines, barbiturates, benzodiazepines, cocaine, opiates, phencyclidine, and THC). Subjects were also excluded for excessive alcohol use (> 20 drinks/week), determined by interview.

This study was approved by the Biomedical Institutional Review Board of the University of Pittsburgh Medical Center.

Nicotine dosing

Discrimination training doses were 20 μg/kg nicotine versus 0 (placebo). Based on plasma nicotine boosts, the 20 μg/kg nicotine dose is comparable to between one-half and one typical cigarette (Perkins et al. 1994a). Nicotine and placebo were provided by a nasal spray delivery procedure developed in our laboratory. This procedure has been found to produce reliable, dose-dependent increases in plasma nicotine (Perkins et al. 1986, 1994b). Use of this or a similar method is particularly advantageous when comparing responses between groups varying in past smoking history, as in this study, since never-smokers cannot readily inhale tobacco smoke (Pomerleau et al. 1989). This method also allows us to examine effects of nicotine per se, isolated from the sensory and other effects of tobacco smoke, and therefore to minimize possible conditioned effects of nicotine via smoke intake in smokers (e.g. associative tolerance; Epstein et al. 1991). Nevertheless, most subjective effects following nicotine intake by this method are comparable to those of nicotine intake via controlled tobacco smoking (Perkins et al. 1994c). Bottles delivered the designated amount of nicotine in saline,

along with peppermint flavoring oil, which was used to mask the taste and smell of nicotine. To equate the placebo and nicotine sprays on immediate sensory effects, the placebo solution contained 60 µl capsaicin (pepper extract), along with peppermint oil, as described previously (Perkins et al. 1994a; 1996a). Each dose was administered in eight sprays (containing eight equally divided doses), with one spray to each nostril every 20 s (total administration time of under 3 min).

Subjective measures

Subjective measures assessed concurrently with behavioral discrimination of nicotine (see below) were the Profile of Mood States (POMS; McNair et al. 1971) and several visual analog scale (VAS) items of specific effects. POMS scales included Tension, Confusion, Fatigue, and Vigor, and the composite scale of Arousal (determined by adding Tension and Vigor and then subtracting Confusion and Fatigue; deWit et al. 1989). VAS subjective effect items ranged from 0 ("not at all") to 100 ("very much") and included "stimulated", "head rush", "relaxed", "dizzy", "alert", "jittery", and (smokers only) "urge to smoke". The POMS scales and most of these VAS items have been shown to be acutely sensitive to nicotine and other drug intake in a dose-dependent fashion (e.g., Perkins et al. 1994b). A separate VAS item, "nasal irritation", assessed peripheral sensory stimuli specific to this nicotine dosing method which might influence the behavioral discrimination of doses.

Nicotine discrimination procedure

The procedure for nicotine discrimination training and generalization testing was adapted from previous research on human drug discrimination (e.g. Preston 1991) and has been described previously in detail (Perkins et al. 1994a, 1996a). Each subject participated in three sessions on 3 separate days: Discrimination Training on day 1, Generalization Testing on day 2, and Nicotine Self-Administration on day 3. On each day, subjects were told that they would receive "a number of different sprays, at least one of which may contain nicotine." No other drugs were mentioned. On days 1 and 2, they were also told they would earn additional bonus money depending on how well they could tell the difference between sprays.

On day 1, subjects were presented with the training doses of 20 µg/kg nicotine and placebo (0) in random order, with one presentation every 25 min. (However, no dose could be presented more than twice consecutively.) The training and placebo doses were labeled "A" and "B", with labeling assignment counter-balanced between subjects within groups. During the first presentation of each, subjects were told which spray they received, "A" or "B" (Discrimination Training). Then, during subsequent presentations in random order, subjects were asked to guess which they received, "A" or "B", by circling the appropriate letter on a form (Discrimination Testing). Subjects then rated the "confidence" with which they made their guess using a 0–100 VAS scale (0 = not at all confident, 100 = extremely confident), prior to receiving feedback on the accuracy of their guess. The criterion for accurate discrimination was at least 80% correct identification of the spray's letter code within ten or fewer testing trials (five for each dose). A minimum of six trials was required (three per dose). Each correct identification was rewarded by adding $1 to their total payment for participation. Subjects discontinued their participation in the session and were discharged from the study after their third incorrect discrimination, since meeting the 80% correct criterion was not possible.

On day 2 (24 h later), subjects correctly discriminating their training dose from placebo on day 1 were again administered the training doses of 0 and 20 µg/kg nicotine in random order, 25 min apart, and instructed to identify each by letter code ("A" or "B") in a continuation of discrimination testing (as in Perkins et al. 1994a). All but one subject (female smoker) correctly identified both. (The subject incorrectly identifying this dose exposure received two additional presentations of each in random order, one every 25 min, and was instructed to identify each in order to demonstrate maintenance of training dose discrimination. Both were correctly identified.) Subjects were then administered a range of doses for Generalization Testing and asked to determine how similar they were to "A" and "B". Doses of 0, 3, 6, 12, and 20 µg/kg nicotine were presented in random order, with 25 min between doses. Subjects were told only that a variety of sprays would be presented and were not told how many different sprays or trials there would be. Subjects saw only one unmarked spray bottle at any one time. Nicotine-appropriate responding was assessed following each dose using quantitative and quantal two-choice behavioral discrimination tasks, also described previously in detail (Perkins et al. 1994a, 1996a). Briefly, the quantitative task consisted of distributing ten "chances" (poker chips) between two sides of a box, with one side labeled "A" and the other "B". Subjects were instructed to place any or all ten chances on either option based on how similar the dose they just received was to "A" and "B". They were also told they would receive 0.25 per "correct" chip placement but were not given feedback on responding. All received the maximum monetary amount ($2.50/trial) after the session, since there was actually no truly correct response for some trials. Nicotine-appropriate responding was defined as the proportion of chances distributed on the side with the same letter code as the nicotine training dose (quantitative measure of discrimination). Following this task, subjects were also asked to make a dichotomous choice between "A" or "B" (quantal measure) and were told that this choice would not be reinforced with money.

Concurrent choice assessment of nicotine self-administration

The concurrent choice procedure of assessing nicotine reinforcement was conducted on day 3, 2–7 days after day 2. This procedure was adapted from that used by others in studying human choice behavior involving other drugs, such as caffeine (Oliveto et al. 1992) and alcohol (deWit and McCracken 1990), and has been described in more detail elsewhere (Perkins et al. 1996b). Briefly, subjects were first presented with separate exposures to the spray bottles containing 0 and 20 µg/kg nicotine (0 and 2.5 µg/kg per spray), clearly identified now by "A" and "B" markings. The experimenter, blind to the dose assigned to bottles, instructed subjects to administer to themselves eight sprays (same as in all prior exposures to spray) from only one of the bottles (A or B, randomly determined), complete subjective effects forms, and then rest quietly for 25 min. They then repeated this procedure for the other spray ("exposure" trials). Subsequently, subjects were instructed to self-administer a total of eight sprays from either or both bottles within a 3-min period ("choice" trial). All self-administrations were done under the observation of the experimenter, who maintained possession of spray bottles at all other times. Subjects repeated this selection of eight sprays within 3 min every 25 min for 2.5 h (total of six choice trials). Nicotine choice was assessed by the number of times subjects selected the nicotine spray (from a total of 48).

General procedures

Discrimination Training and Testing (day 1) and Generalization Testing (day 2) occurred on consecutive days to minimize loss of discrimination ability prior to generalization. Nicotine self-administration (concurrent choice, day 3) occurred within 1 week after Generalization Testing. In all sessions, the experimenter was kept blind to subject's dosing schedule ("A" and "B"; the five doses for generalization testing on day 2 were labeled "C" through "G" for the experimenter). (However, it was not practical to keep experimenters blind to subjects' smoking status.) Subjects were instructed to remain abstinent overnight from smoking (determined by expired-air CO < 13 ppm) prior to each morning session.

Each day, subjects were first instructed to remain quiet for 10 min while resting in a comfortable armchair. A baseline assessment of each subjective measure was then obtained. Subsequently, subjects engaged in the Discrimination Training/Testing (day 1) or Generalization (day 2) phases of the Discrimination Procedure. Each subjective measure was completed once between 4 and 5 min after each dose administration, followed by behavioral discrimination at 6 min post-dosing. Subjects rested quietly until the next trial, approximately 25 min later.

On day 2, following the last trial, a single blood sample was obtained by venipuncture from each subject to gauge nicotine exposure. Based on recent research (Benowitz and Jacob 1993; Perkins et al. 1994b), it was likely that plasma nicotine levels would be slightly lower in never-smokers versus smokers, the *opposite* of that expected if dispositional (kinetic) tolerance was present. In any case, these samples were used to eliminate reduced exposure as an explanation for attenuated discrimination in smokers. Each sample was collected into an EDTA tube, spun down to separate plasma, and stored at −60° C for later analysis. Plasma nicotine concentration was determined in the laboratory of Drs Neal Benowitz and Peyton Jacob III by gas chromatography with nitrogen-phosphorus detection using 5-methylnicotine as the internal standard (Jacob et al. 1981).

On day 3, subjects engaged in the concurrent choice procedure involving self-administration of eight nicotine and/or placebo sprays every 25 min for 2.5 h, as described above.

Data analyses

Trials to criterion (≥ 80% correct) during the acquisition of training dose discrimination (day 1) was analyzed by *t*-test between smokers versus never-smokers. Confidence ratings during these acquisition trials were analyzed by analysis of variance (ANOVA) of smoking status and sex. Significant ANOVAs were followed up by comparisons using Fisher's least significant differences *t*-test (Huitema 1980). Nicotine-appropriate responding during the quantitative assessment of generalization (day 2) was analyzed by regression analyses using Generalized Estimating Equations (GEE; Karim and Zeger 1988). GEE provides regression coefficients for effects of interest (smoking status, nicotine generalization dose, subject sex), estimate of variance, and a *t*-test of significance. This procedure controls within-subject correlation among responses across doses, a violation of assumptions for ANOVA, and is more appropriate than ANOVA for "count" data such as our measure of nicotine-appropriate responding (i.e. number of chips out of ten in "nicotine" side of box). Similarly, logistic regression using GEE was employed to analyze the results of quantal (all-or-none) responding during generalization trials. Direct relationships between nicotine-appropriate behavior and responses on subjective effects were examined by stepwise multiple regression to determine whether any of these effects were associated with behavioral discrimination of nicotine after excluding any peripheral sensory effects of nasal irritation. Nicotine self-administration on the concurrent choice day (day 3) was analyzed by ANOVA of smoking status and sex. Subjective responses to nicotine during initial training dose exposure on day 1 were calculated as the difference between effects of nicotine versus placebo trials. These effects of nicotine per se were related to nicotine self-administration on day 3 by correlation in an attempt to see if initial responses might predict magnitude of self-administration. Statistical significance of effects was determined by $P < 0.05$, while effects at $P < 0.10$ but > 0.05 were deemed marginally significant.

Results

No smoker was dropped from the study for failure to meet the CO requirement indicating overnight smoking abstinence. COs of smokers upon arrival for sessions ranged from 5 to 12 ppm.

Acquisition of training dose discrimination

Only one subject (a female smoker) failed to reliably discriminate the training doses of 0 versus 20 μg/kg nicotine on day 1 and did not continue in the study. Data from this subject were excluded from analyses. Smokers and never-smokers acquired discrimination of the training doses in a similar number of trials on day 1 (6.4 ± 0.2 versus 6.0 ± 0.0, respectively), as only two smokers required more than the minimum of six test trials (both met criterion in eight trials). Consequently, there was also no significant difference between smokers and never-smokers in percent correct identification during acquisition (94% versus 98%, respectively). Despite successfully learning to discriminate the training doses, smokers were significantly less confident than never-smokers in their identifications of each during discrimination testing (72.2 ± 7.8 versus 89.0 ± 3.3, respectively, on the 0–100 scale) [$F(1,17) = 7.48$, $P < 0.02$]. Interestingly, the smoking status × sex interaction was significant [$F(1,17) = 9.37$, $P < 0.01$], as this effect was due to particularly low confidence among female smokers (52.3 ± 11.8), compared with male smokers (88.8 ± 3.1), male never-smokers (86.6 ± 5.8), and female never-smokers (91.3 ± 3.7). Smokers also acquired discrimination nearly as readily as never-smokers, despite significantly smaller responses to the nicotine training dose on the following subjective measures: VAS scales of "dizzy" ($t = 2.27$, $P < 0.05$), "head rush" ($t = 4.36$, $P < 0.001$), and decline in "alert" ($t = 2.63$, $P < 0.02$). There were no differences between smokers and never-smokers in effects of nicotine on the POMS scales or in nasal irritation. Of all the subjective scales, only POMS-Tension ($P < 0.05$) and VAS items of "dizzy" ($P < 0.05$) and "jittery" ($P < 0.01$) were significantly different (higher) at baseline in smokers compared with never-smokers, suggesting that withdrawal relief by nicotine did not influence smokers' acquisition of discrimination.

Generalization across doses

GEE analyses revealed that nicotine-appropriate responding was highly related to generalization dose (variance estimate = 0.51, $t = 31.08$, $P < 0.001$), as expected. Responding was also influenced by the interaction of smoking status × dose (estimate = 0.30, $t = 3.35$, $P < 0.01$), but the main effect of smoking status was not significant. As shown in Fig. 1 (top), nicotine-appropriate responding on the quantitative measure of discrimination was attenuated for smokers versus never-smokers at 20 μg/kg, but there was no difference at lower doses or placebo. Results for the quantal measure of discrimination were similar, with significant

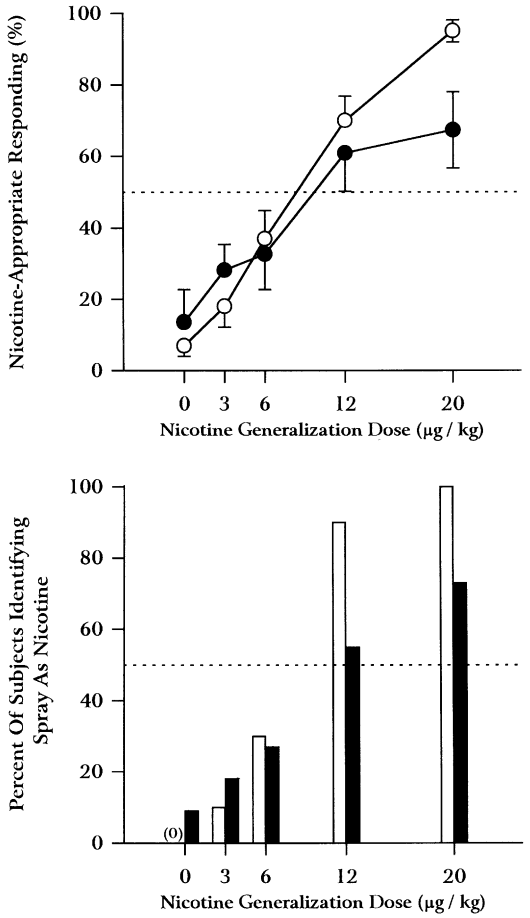

Fig. 1 Quantitative (*top*) and quantal (*bottom*) measures of generalization of discrimination across nicotine generalization doses in smokers ($n = 11$) versus never-smokers ($n = 10$). *Dotted line* indicates 50% or chance responding. Group differences in responding were observed at 20 μg/kg for quantitative and 12 μg/kg for quantal responding. □, –○– Never-smokers; ■, –●– smokers

effects of dose ($t = 5.12$, $P < 0.001$) and group × dose ($t = 4.13$, $P < 0.001$), as also shown in Fig. 1 (bottom). However, chi-square analysis of responding at each generalization dose indicated marginally different responding between smokers and never-smokers at 12 μg/kg ($\chi^2 = 3.47$, $P < 0.10$) but no difference at 20 μg/kg. The nicotine-appropriate quantal response was made at the 12 μg/kg dose by six of 11 smokers and nine of ten never-smokers. There was no significant correlation of nicotine-appropriate responding on the quantitative task with FTND score ($r = -0.12$) or cigarettes/day ($r = 0.02$), indicating that severity of nicotine dependence was unrelated to nicotine discrimination in this homogeneous and small sample of young smokers.

GEE analyses also revealed an interaction of dose × group × sex for the quantitative ($t = 2.16$, $P < 0.05$) and quantal ($t = 1.98$, $P < 0.06$) measures of generalization, although there was no main effect or other interactions involving sex. As shown in Fig. 2, placebo (0) engendered more nicotine-appropriate responding in female smokers compared with never-smokers and male smokers. Consequently, their responding was flatter across lower doses and less sensitive with increasing nicotine generalization dose.

Plasma nicotine levels at the end of the generalization testing session were 9.5 ± 1.2 versus 7.8 ± 0.6 ng/ml for smokers versus never-smokers, respectively ($t = 1.30$, NS). Because plasma levels were slightly higher in smokers, as previously observed (Benowitz and Jacob 1993; Perkins et al. 1994b), this rules out the possibility that their attenuated discrimination was due to reduced blood levels.

Nicotine self-administration

Nicotine self-administration on day 3 was greater for smokers versus never-smokers (no. of nicotine sprays = 17.5 ± 4.1 versus 4.6 ± 2.5, respectively) [$F(1,17) = 6.99$, $P < 0.02$]. Self-administration by smokers was not significantly different from chance (50%), while self-administration of never-smokers was significantly less than chance ($t = 4.08$, $P < 0.01$), suggesting avoidance of nicotine. The range of nicotine sprays on day 3 was 4–41 for smokers, with three of 11 self-administering more than 24 (i.e. >50%), and 0–20 for never-smokers, with six of ten self-administering 0 nicotine sprays. There was no systematic pattern of nicotine spray self-administration across trials, as smokers self-administered 2.7 ± 0.9 nicotine sprays on trial 1 versus 2.8 ± 0.9 on trial 6, while never-smokers self-administered 0.8 ± 0.4 on trial 1 and 1.0 ± 0.7 on trial 6. Nicotine self-administration tended to be lower in women versus men among smokers (10.8 ± 3.8 versus 23.0 ± 6.2, respectively, $t = 1.89$, $P < 0.10$), and non-significantly lower in women versus men among never-smokers, (1.6 ± 1.2 versus 7.6 ± 4.7, respectively).

For smokers, the following subjective responses to initial nicotine training dose (20 μg/kg) exposure during day 1 were significantly or marginally related to self-administration of nicotine on day 3: VAS scales of "dizzy" ($r = 0.58$, $P < 0.05$), "jittery" ($r = 0.53$, $P < 0.05$), "relaxed" ($r = -0.50$, $P = 0.06$), "stimulated" ($r = 0.68$, $P = 0.01$), and "head rush" ($r = 0.49$, $P = 0.06$), and the POMS scale of Tension ($r = 0.49$, $P = 0.06$). Thus, greater responses to nicotine suggesting subjective stimulation were associated with greater subsequent nicotine self-administration in smokers. As with discrimination, there was no significant relationship between self-administration and Fagerstrom score or number of cigarettes per day. For never-smokers, no subjective responses to initial nicotine exposure were significantly associated with subsequent self-administration.

Subjective and behavioral responses associated with discrimination

Nicotine-appropriate responding during the generalization test (day 2) was associated with concurrent

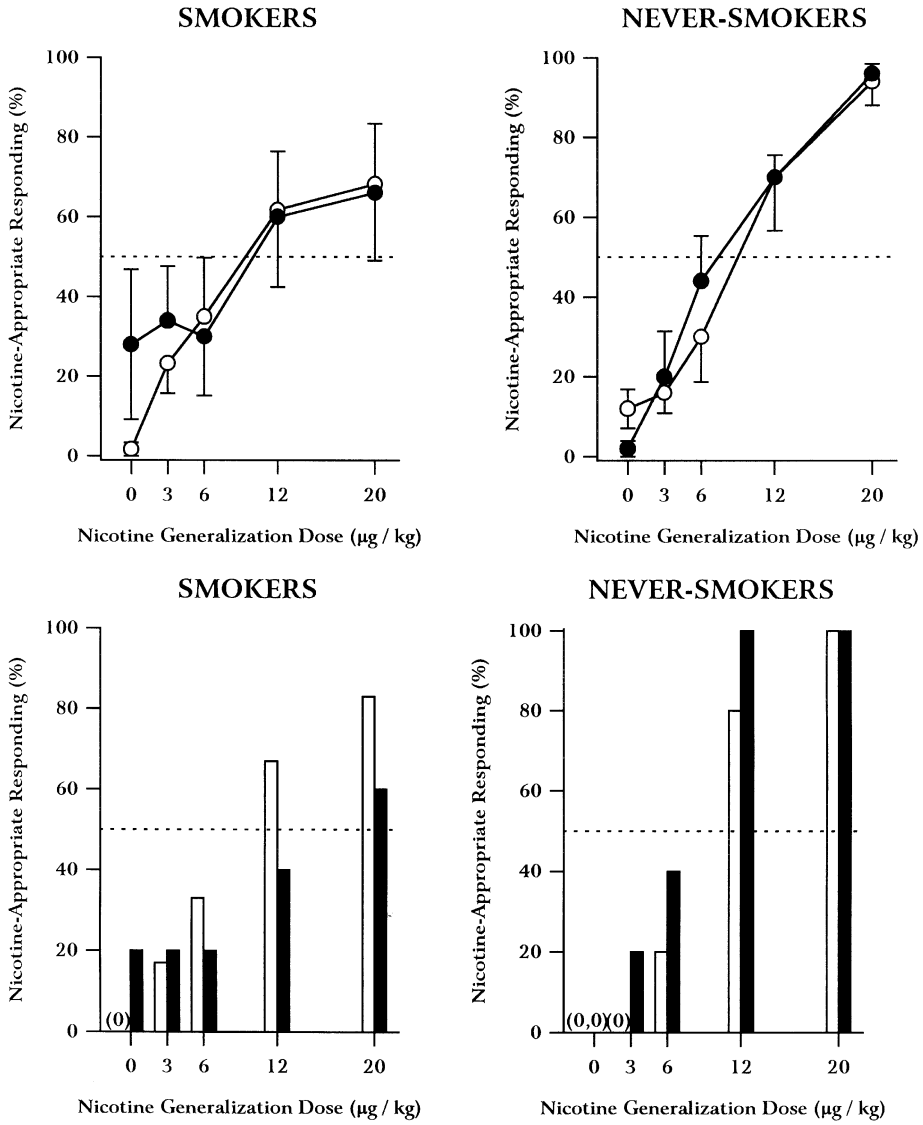

Fig. 2 Quantitative (*top*) and quantal (*bottom*) measures of generalization of discrimination across nicotine generalization doses by subject sex for each smoking status group. □, –○– Males; ■, –●– females

responses on the VAS scales of "head rush" (in smokers and nonsmokers) and nasal irritation (in smokers only), but no other subjective effects. Regression analyses determined that, after removing the effect of irritation, "head rush" remained associated with nicotine-appropriate responding in both smokers ($P < 0.10$) and never-smokers ($P < 0.001$). ANOVA results also indicated significant nicotine dose effects on "head rush" [$F(4,76) = 10.86$, $P < 0.001$] and nasal irritation [$F(4, 76) = 5.01$, $P < 0.01$] but a significant interaction of dose × smoking status only for "head rush", [$F(4,76) = 4.87$, $P < 0.01$] (see Fig. 3). There was no main effect of smoking status on either measure. Compared with never-smokers, smokers had attenuated responses on "head rush" at 20 µg/kg ($P < 0.001$) and 12 µg/kg nicotine ($P < 0.10$), consistent with their attenuated nicotine discrimination, as well as attenuated irritation at 20 µg/kg nicotine ($P < 0.01$). Similar to previous research (Perkins et al. 1994a), significant main effects of nicotine generalization dose were also observed for VAS scales of "jittery", "relaxed" (decrease), and "stimulated", and for POMS scales of Tension (increase), Vigor (decrease), Fatigue (increase), and Arousal (decrease), but none of these was associated with nicotine-appropriate responding.

The relationship of nicotine self-administration on day 3 to nicotine-appropriate responding at 20 µg/kg on day 2 was significant for never-smokers ($r = -0.54$, $P = 0.05$) but not for smokers ($r = 0.28$). Thus, greater nicotine-appropriate responding at the highest dose was associated with reduced self-administration of that same spray versus placebo for never-smokers.

Discussion

The discriminative stimulus effects of nicotine were attenuated at higher doses, particularly the training dose of 20 µg/kg, in smokers compared with never-smokers. These results suggest that smokers may

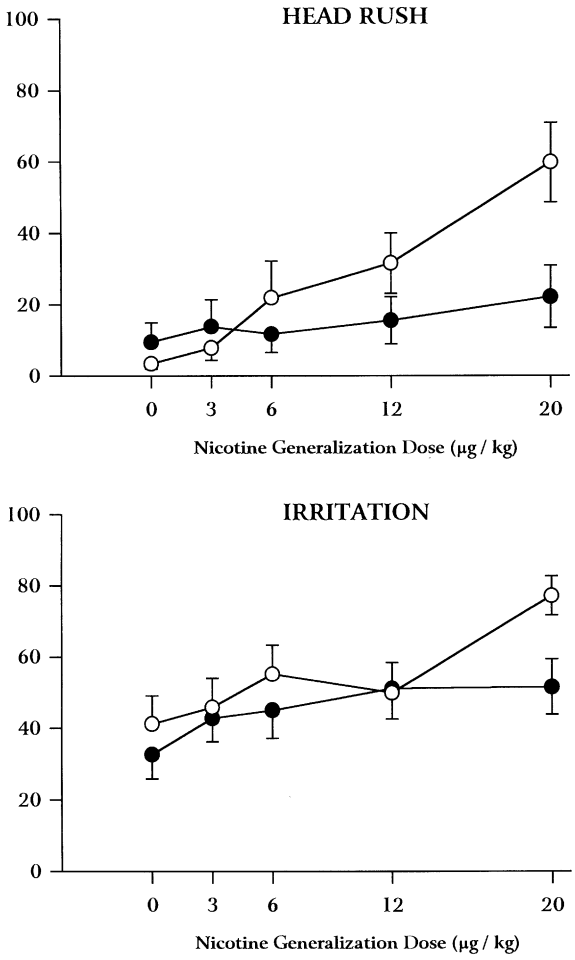

Fig. 3 Subjective "head rush" and nasal irritation responses across nicotine generalization doses in smokers versus (–●–) never-smokers (–O–)

become tolerant to nicotine's discriminative stimulus effects through chronic exposure to nicotine from tobacco smoking. Thus, relative to never-smokers, smokers require greater amounts of nicotine to discriminate nicotine. Nevertheless, although smokers had smaller selected subjective responses to initial nicotine exposure, further suggesting tolerance, they generally did not differ from never-smokers in the rate at which they were able initially to acquire discrimination of training doses on day 1. It is possible that the subjective measures on which smokers showed attenuated responding are not relevant to nicotine discrimination or that this attenuated responding was still of sufficient magnitude as to allow for clear discrimination of stimulus effects by smokers. Smoking status influenced discrimination behavior only after exposure to higher nicotine generalization doses on day 2. A difference in generalization responding (or perhaps maintenance of discrimination), therefore, was not associated with a difference in acquisition, suggesting these processes may be somewhat different.

It is important to note that the apparent tolerance to nicotine discrimination observed here in smokers may *under* estimate the magnitude of tolerance typically found in most smokers when consuming nicotine via more common means, through tobacco smoking. First, stimuli accompanying smoking (e.g. taste and smell of smoke) may produce associative tolerance to nicotine's stimulus effects through smoking (Epstein et al. 1991), over and above non-associative, pharmacological tolerance to these effects. Use in this study of nicotine by nasal spray, a delivery system equally unfamiliar to smokers and never-smokers, presumably eliminated the influence of associative tolerance in smokers. Second, although smokers in this study had relatively high FTND scores, suggesting dependence, they were also much younger than most dependent smokers. Examination of discrimination between older smokers versus never-smokers could reveal greater tolerance due to lengthier tobacco smoking history. Third, the slightly higher plasma nicotine levels in smokers versus never-smokers may have narrowed the differences between groups, and equating biological exposure between groups may reveal somewhat greater effects of smoking status on discrimination and other effects of nicotine.

In any case, despite a significant difference in discrimination of 20 μg/kg nicotine between smokers and never-smokers, this difference appeared less robust than a previously observed difference in discrimination across a range of doses between two groups of smokers differing only in their respective training doses (Perkins et al. 1996a). This observation serves to highlight the point that acute situational factors, such as training conditions, can exert as large (or larger) an effect on drug discrimination responding as that due to differences in chronic history of drug exposure.

Although the quantitative and quantal measures of nicotine generalization produced generally similar findings, specific results indicated attenuated generalization responding of smokers only at 20 μg/kg for the quantitative procedure and only at 12 μg/kg for the quantal procedure. This may suggest that these two procedures are differentially sensitive to differences in tolerance to specific doses of nicotine between smokers and never-smokers. However, given the relatively small sample size in this study, this may be a chance finding, and little can be concluded regarding how these observations may contribute to our understanding of tolerance to nicotine discrimination. These findings would also seem to have little to contribute toward resolving whether drug discrimination is a continuous or quantal process (e.g., Barrett et al. 1994).

Nicotine discrimination was associated with greater self-report of "head rush" following nicotine intake, consistent with previous studies using similar procedures (Perkins et al. 1994a, 1996a) and indicating that

this effect may be salient in "guiding" discrimination behavior for both smokers and never-smokers. Relative to never-smokers, "head rush" and nicotine-appropriate responding were both attenuated in smokers while self-administration was greater, perhaps consistent with the notion that tolerance promotes greater drug intake (Perkins et al. 1994b). Furthermore, within never-smokers, nicotine-appropriate responding was inversely associated with nicotine self-administration, indicating that reduced sensitivity to the discriminative stimulus effects of nicotine leads to greater nicotine intake. However, no significant relationship between discrimination and self-administration was seen in smokers. The relatively small and homogeneous sample of smokers may have reduced our ability to more conclusively examine relationships among discrimination, self-administration, and subjective responses to nicotine within smokers. Examination of these effects across a broader range of smokers (e.g. including "chippers"; Shiffman 1989) may reveal a similar inverse relationship between discrimination and self-administration. Nevertheless, these results suggest that the discriminative stimulus effects of nicotine are fairly pronounced in naive individuals upon initial exposure to nicotine, such as when teens experiment with tobacco. With continued use, though, the magnitude of these effects may recede, perhaps leading to the commonly observed escalation in tobacco use (McNeill 1991) in order to maintain discrimination of effects.

Although tobacco withdrawal was not formally assessed, it is unlikely that withdrawal relief due to nicotine spray enhanced smokers' acquisition or generalization of nicotine discrimination. There were few baseline differences in subjective measures between smokers and never-smokers, and only "head rush", a measure not different at baseline nor one typically associated with withdrawal, was associated with discrimination behavior. However, inclusion of a broader array of subjective measures, including formal withdrawal scales, and examination of different durations of abstinence could reveal an influence of withdrawal severity on enhancing nicotine discrimination in smokers. It would also be important to examine change in nicotine discrimination after smoking cessation, since some research suggests that tolerance recedes quickly after removal of nicotine exposure (Lee et al. 1987). Reinstatement of greater magnitude of discriminative stimulus effects of nicotine after cessation could be critical to explaining relapse upon exposure to small amounts of nicotine (Kenford et al. 1994; see also Chiamulera et al. 1996).

On the other hand, although smokers appeared to be tolerant to the subjective and discriminative stimulus effects of 20 μg/kg nicotine, there was no difference between smokers and never-smokers in nicotine-appropriate responding at lower nicotine doses. It is conceivable, therefore, that *threshold* for discrimination (i.e. lowest discriminable dose) may not differ between smokers and never-smokers. This would suggest that sensitivity to stimulus effects of low nicotine doses does not change with continued nicotine exposure via smoking (i.e. tolerance does not develop to low dose effects). However, since responding across generalization doses is dependent upon the specific training dose employed (Perkins et al. 1996a), lack of difference in responding between smokers and never-smokers at these lower doses may be specific to the use of this training dose. Formal test of acquisition of nicotine discrimination at lower doses is needed to determine whether chronic tolerance may develop to discrimination threshold.

As suggested, reduced sensitivity of smokers to these effects indicates presence of chronic tolerance to nicotine. Yet, among smokers, greater responses on subjective measures associated with "stimulation" during initial nicotine training dose exposure on day 1 were associated with greater nicotine self-administration on day 3. This finding is consistent with previous studies of self-administration (Perkins et al. 1996b, 1997) and with animal research showing that greater behavioral activation following nicotine exposure was associated with greater subsequent nicotine place preference in mice (Shechter et al. 1995). These observations would seem contrary to the notion that greater tolerance is associated with greater self-administration. However, although nicotine discrimination behavior was unrelated to self-administration among smokers, it is possible that those smokers who did *not* self-administer nicotine were those most tolerant to the "stimulating" effects of the relatively low nicotine dose per spray (2.5 μg/kg). These effects may therefore have been too subtle to maintain self-administration in the most tolerant smokers. In contrast, those smokers who did self-administer nicotine spray may have been less tolerant, experiencing sufficient "stimulation" to maintain self-administration. Yet, it was not the case that smokers self-administering more nicotine were less nicotine dependent, as Fagerstrom score and number of cigarettes per day were unrelated to self-administration. Furthermore, as with nicotine discrimination behavior, withdrawal relief did not appear to influence nicotine self-administration in smokers since increases, rather than decreases, in subjective responses to nicotine on measures such as POMS-Tension and VAS "jittery" (along with decreased "relaxed") were associated with nicotine self-administration.

Nicotine self-administration was significantly below 50% in never-smokers, indicating that nicotine by nasal spray was aversive. This observation is similar to that of Hughes et al. (1989), who found greater aversion to nicotine gum in never-smokers versus smokers. Smokers in their study also did not self-administer nicotine more than 50%. One possible explanation for the very low self-administration of nicotine by never-smokers could be that, as with "head rush" and other stimulus effects of nicotine, never-smokers are not as

tolerant to aversive effects of nicotine when compared with smokers. This possibility is only modestly supported by this study, since the only differences in subjective responses to the nicotine training dose were never-smokers' greater "dizzy" and "head rush" and decline in "alert"; there were no differences on scales more clearly reflecting aversive effects, such as POMS-Tension, Confusion, and decreased Fatigue, or VAS "jittery", or in nasal irritation.

Similarly, because humans largely self-select their smoking status, it is conceivable that those who become regular smokers differ from those who remain never-smokers in ways (other than their smoking history) which influence their perception of the discriminative stimulus effects of nicotine (e.g. genetic differences, covarying factors such as other environmental experiences). Smokers and never-smokers in this study were similar in typical alcohol intake, ruling out this important alternative explanation (Collins et al. 1988). Further study will be required to tease apart whether tolerance or stable individual differences are responsible for these differences due to smoking status.

Discrimination behavior appeared to be less sensitive to nicotine dose in women smokers compared with the other subgroups. In particular, maintenance of placebo discrimination from training to generalization (i.e. day 1 to day 2) was poorer in women smokers, as previously observed (Perkins et al. 1996a). Women smokers were also significantly less confident than men smokers during the discrimination testing phase of acquisition on day 1, and the only subject unable to acquire discrimination of training doses was a female smoker. Nicotine self-administration also tended to be reduced in women versus men on day 3. These findings are consistent with other evidence that women appear to be less sensitive to some effects of nicotine and that nicotine is less reinforcing in women than men (Perkins 1996).

In summary, smokers were tolerant to the discriminative stimulus effects of nicotine at the highest dose. They were also tolerant to subjective effects of nicotine, including "head rush", which was associated with discrimination responding in smokers and never-smokers. Further, smokers self-administered greater amounts of nicotine, consistent with the notion that tolerance to the discriminative stimulus effects of nicotine may lead to greater self-administration and the onset of dependence, although discrimination and self-administration were related only within never-smokers and not within smokers. Compared with men smokers, women smokers tended to self-administer less nicotine and were less confident in discriminating the training doses. Future research should examine other stable subject characteristics associated with nicotine discrimination, such as history of other drug use (Collins et al. 1988), as well as acute situational factors that may alter discrimination (e.g. acute drug use; Kim and Brioni 1994).

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