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Cue dependency of nicotine self-administration and smoking

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Abstract

A paradox exists regarding the reinforcing properties of nicotine. The abuse liability associated with smoking equals or exceeds that of other addictive drugs, yet the euphoric, reinforcing and other psychological effects of nicotine, compared to these other drugs, are more subtle, are manifest under more restricted conditions, and do not readily predict the difficulty most smokers experience in achieving abstinence. One possible resolution to this apparent inconsistency is that environmental cues associated with drug delivery become conditioned reinforcers and take on powerful incentive properties that are critically important for sustaining smoking in humans and nicotine self-administration in animals. We tested this hypothesis by using a widely employed self-administration paradigm in which rats press a lever at high rates for 1 h/day to obtain intravenous infusions of nicotine that are paired with two types of visual stimuli: a chamber light that when turned on signals drug availability and a 1-s cue light that signals drug delivery. We show that these visual cues are at least as important as nicotine in sustaining a high rate of responding once self-administration has been established, in the degree to which withdrawing nicotine extinguishes the behavior, and in the reinstatement of lever pressing after extinction. Additional studies demonstrated that the importance of these cues was manifest under both fixed ratio and progressive ratio (PR) schedules of reinforcement. The possibility that nicotine-paired cues are as important as nicotine in smoking behavior should refocus our attention on the psychology and neurobiology of conditioned reinforcers in order to stimulate the development of more effective treatment programs for smoking cessation.

Keywords: Nicotine; Smoking; Addiction; Drug-cues; Conditioned drug response; Drug reinforcement

1. Introduction

Tobacco smoking is the most important preventable cause of death in developed countries and is rapidly becoming a significant health problem in developing countries. Although the prevalence of smoking has gradually declined among adults (but not teens) in developed countries, worldwide consumption of tobacco is still rising. It is predicted that about 3 million smokers worldwide die annually from smoking, and that the rapid increase in smoking in developing countries (Peto et al., 1996) will cause this toll to rise to about 10 million annually by the year 2030. Several hundred million adults who are current smokers are expected to die from smoking (Peto et al., 1996). Although the harmful health effects from smoking are widely known, only an estimated 3% of smokers successfully quit each year, less than 10% of those who attempt smoking abstinence (Shiffman et al., 1998).

With these risks associated with smoking, it is relevant to question why people smoke, why they develop an addiction to smoking, and why many of them cannot quit? The widely accepted answers are that they smoke because smoking is a very efficient way of rapidly delivering nicotine to the brain, nicotine has powerful reinforcing effects like cocaine, heroin and other drugs of abuse, and like those other drugs, chronic usage leads to dependence and addiction (Benowitz, 1996; Stolerman and Shoaib, 1991). Based on the very reasonable assumption that people smoke tobacco primarily to obtain nicotine, much animal research has focused on the

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neurophysiological consequences of nicotine and pharmacological treatments that can reduce its effects (Balfour et al., 2000; Di Chiara, 2000). In the human clinical literature, a similar emphasis has been placed on the development of strategies for reducing cigarette smoking, such as nicotine gum or patch, that are directed at the actions of nicotine (Benowitz, 1993; Rose and Corrigall, 1997).

However, a paradox exists in both the animal and human literature that is not adequately addressed by these approaches. The abuse liability, frequency of consumption, and rate of relapse associated with smoking are at least equal to other addictive drugs such as stimulants or opiates (Anthony et al., 1994). In contrast, the euphoric, reinforcing and other psychological effects of nicotine, compared to other drugs, are more subtle, are manifested under more restricted conditions, and do not readily predict the difficulty most smokers experience in achieving abstinence (Goldberg et al., 1981; Rose and Corrigal, 1997; Rose and Levin, 1991). The limited success rates of smoking cessation treatments that are directed at nicotine's pharmacological actions (Balfour and Fagerstrom, 1996) also suggest that nonnicotine factors may also be involved in maintaining smoking behavior and fostering relapse.

One possible resolution to this paradox is that in addition to its intrinsic reinforcing properties, nicotine is particularly effective in establishing or magnifying the incentive properties and reinforcing effects of associated, nonpharmacological stimuli, such as the predictive visual and/or auditory cues typically found in animal self-administration paradigms, or the taste and smell of tobacco smoke for smokers (Balfour et al., 2000; Di Chiara, 2000; Goldberg and Henningfield, 1988; Goldberg et al., 1981; Perkins et al., 2001a; Rose and Corrigall, 1997; Rose and Levin, 1991). Smoking may be a more effective way of establishing these stimuli as conditioned reinforcers, compared to other routes of nicotine administration (e.g., smokeless tobacco) that are likely to be associated with fewer salient environmental cues and, therefore fewer discrete pairings of cues with nicotine delivery (i.e., inhaled versus absorption through the mucous membrane).

In the present paper, we will briefly review the results of human and animal research in support of the hypotheses that: (a) nicotine is the major psychoactive factor in tobacco that drives smoking behavior; (b) the primary (unconditioned) reinforcing effects of nicotine, when considered in isolation, are inadequate to explain the tenacity of smoking behavior or the only modest success of nicotine-based treatment strategies; and (c) environmental stimuli associated with exposure to nicotine play a critical role in maintaining drug-taking behavior and in promoting relapse. We will then present new studies from our laboratory that test the hypothesis regarding the importance of drug-related environmental cues in an animal model of nicotine reinforcement. Our results indicate that the high rate of nicotine self-administration exhibited by rats, which has been reported by several laboratories including our own, is heavily dependent on environmental cues associated with the drug.

2. Nicotine as the major factor driving smoking behavior

Nicotine is the primary constituent of tobacco that reinforces smoking behavior (Stolerman and Jarvis, 1995). The few attempts made by tobacco companies to market nonnicotine tobacco cigarettes have generally failed (Jaffe, 1990), indicating that nicotine is necessary (but perhaps not always sufficient) in promoting cigarette smoking behavior in most individuals. Self-administration of nicotine in isolation from tobacco smoke has been demonstrated in smokers not trying to quit, using intravenous (Henningfield and Goldberg, 1983), oral (Hughes et al., 2000) and nasal (Perkins et al., 1996) routes of administration. As expected, nicotine self-administration is greater in smokers than in nonsmokers or exsmokers (Hughes et al., 2000; Perkins et al., 1997, 2001b), and in briefly abstinent versus non-abstinent smokers (Perkins et al., 1996). Notably, some of this research suggests that it is the pleasurable subjective mood effects of nicotine, rather than withdrawal relief, that promotes nicotine self-administration when tested in smokers who were not trying to quit (Perkins et al., 1996). Pretreatment with nicotine administered in rapid bolus form, as in smoking, attenuates subsequent smoking behavior in dose–response fashion (e.g., Perkins et al., 1992). However, this attenuation is not as complete as would be expected if nicotine intake were the only factor influencing smoking behavior. This common observation suggests that, in addition to its delivery of nicotine, smoking is reinforced by other nonnicotine characteristics, a notion that will be explored throughout this review.

Despite the growing body of research with humans, the effects of nicotine per se have been far more thoroughly examined in nonhuman animal models. Most of the research on the mechanisms of nicotine's actions has used either experimenter-controlled administration of drug to laboratory animals or in vitro preparations. These studies have established that nicotine's physiological and behavioral effects are the result of its complex, agonistic actions on a family of nicotinic acetylcholine receptors (nAChRs) that differ in terms of their α and β subunit composition, cellular localization, topographic distribution throughout the brain, interaction with different neurotransmitters systems and their functional state (Changeux et al., 1984, 1998; Dani and Heinemann, 1996; Di Chiara, 2000). There are two aspects of this rich literature that are particularly relevant to the present topic. Acute exposure of these receptors to nicotine produces activation rapidly followed by desensitization; more prolonged exposure results in an increase in receptor number, which is thought to reflect a compensation for long-lasting inactivation of those receptors (Dani and Heinemann, 1996; Marks et al., 1983, 1992; Wonnacott, 1990). Receptors that differ in subunit

composition also differ in the extent to which exposure to nicotine results in desensitization and inactivation, and in the extent and rate of recovery to a functional state after nicotine is withdrawn (Alkondon and Albuquerque, 1993; Fenster et al., 1997; Hsu et al., 1996; Ke et al., 1998; Vibat et al., 1995). These cycles of nicotine-induced receptor activation, desensitization and more prolonged inactivation, with chronic nicotine treatment, and reactivation with nicotine withdrawal must be taken into account if we are to understand the role of sustained nicotine exposure in maintaining smoking behavior.

A second important discovery of relevance to the present discussion is that nicotine, like other drugs of abuse such as psychomotor stimulants, increases the release of dopamine from terminals fields of the mesolimbic dopamine system (Balfour et al., 2000; Di Chiara, 2000). It is believed that activation of this system is a critical component of the reinforcing and addictive properties of abused drugs (Koob et al., 1998; Robinson and Berridge, 1993; Wise and Bozarth, 1987), including nicotine (Balfour et al., 2000; Di Chiara, 2000).

As stated above, considerable knowledge has been gained about nicotine's mechanisms of action using experimenter-administered methods of drug delivery. However, the utility of an animal model is predicated on its ability to incorporate essential features of human phenomenon that it is modeling in a way that permits systematic investigation of those features. For this reason, investigators who study the neurobiological mechanisms of drugs such as opiates, stimulants and alcohol, among others, are making increasing use of self-administration models in which drug administration is contingent on the animal's behavior, as a way to more closely mimic the manner in which drugs of abuse are experienced by humans (Bozarth et al., 1989; Carroll et al., 1990; Johanson and Schuster, 1981; Roberts and Richardson, 1992; Shaham and Stewart, 1995). It could be argued that, while the drug self-administration model has a certain face validity, it does not necessarily follow that the information yielded will be different in any important way from that obtained by noncontingent models, other than as it applies specifically to the self-administration behavior itself. However, there is mounting evidence that self-administered and experimenter-controlled methods of drug delivery can produce very different effects on basic physiological processes, indicating that one or more factors of potentially critical importance in understanding the dynamics of drug abuse are missing in the noncontingent model (Ator and Griffiths, 1993; Donny et al., 1999a, 2000a; Dworkin et al., 1995; Kiyatkin et al., 1993; Moolten and Kornetsky, 1990; Smith et al., 1982; Stefanski et al., 1999; Wilson et al., 1994).

While some investigators have questioned whether nicotine could serve as a powerful reinforcer in such an animal model (Bozarth and Pudiak, 1996; Dworkin et al., 1993), there is now overwhelming evidence for its effectiveness in reinforcing operant responding in a variety of species, including nonhuman primates (Goldberg et al., 1981; Slifer and Balster, 1985; Wakasa et al., 1995), dogs (Risner and Goldberg, 1983), rats (Chiamulera et al., 1996; Corrigall, 1992, 1999; Corrigall and Coen, 1989; Donny et al., 1995, 1998, 1999b, 2000a, 2000b; Lynch and Carroll, 1999; Shaham et al., 1997; Shoaib and Stolerman, 1999; Shoaib et al., 1997, 1999; Smith and Roberts, 1995; Tessari et al., 1995; Valentine et al., 1997; Watkins et al., 1999) and mice (Epping-Jordan et al., 1999; Martellotta et al., 1995; Picciotto et al., 1998; Stolerman et al., 1999). The range of species, including humans (Henningfield and Goldberg, 1983), that find nicotine reinforcing speaks to the generality of the phenomenon. Like opiates and stimulants, some of the effects of nicotine are also different depending on whether the drug is self- or noncontingently administered (Donny et al., 1999a, 2000a).

Studies utilizing self-administration have verified some of the conclusions drawn from noncontingent (experimenter-controlled) models that were noted above. For example, the upregulation of brain nAChR binding sites, reported after prolonged experimenter-administered nicotine in rats, has also been found after nicotine self-administration (Donny et al., 2000b). Similar to other addictive drugs, the reinforcing effects of nicotine appear to depend on direct and/or indirect stimulation of mesolimbic dopamine neurons, since lesions or pharmacological blockade of these cells attenuate nicotine self-administration (Corrigall, 1999; Corrigall and Coen, 1991), and the pattern of increased immediate-early gene expression exhibited in the terminal fields of dopamine cells is similar for cocaine and nicotine self-administration in rats (Pagliusi, 1996; Pich et al., 1997). These effects are likely to be mediated in large part by nicotine's actions on nAChRs containing the $\beta 2$ subunit since mutant mice without this receptor subunit will lever-press for cocaine and food but not nicotine (Epping-Jordan et al., 1999; Picciotto et al., 1998).

3. The need to postulate a role for nonpharmacological stimuli in nicotine self-administration and smoking

As noted, nicotine is the principal pharmacological ingredient in cigarette smoke driving smoking behavior, and it can serve as a reinforcer for self-administration behavior in animals. However, other evidence makes it difficult to explain several key features of smoking and nicotine self-administration by simply invoking the primary (unconditioned) reinforcing effects of nicotine. These features include the relative insensitivity of smoking and nicotine self-administration to dose, the tenacity of these behaviors in the face of reduced nicotine stimulation caused by either absence of the drug or reduced sensitivity of the underlying receptor systems, the observation that smoking behavior is only partly attenuated by nicotine pretreatment and the limited success of nicotine-based treatment strategies for smoking cessation.

Both smoking in humans and intravenous nicotine self-administration in humans and animals are relatively independent of changes in dose in the middle of the effective dose range (Di Chiara, 2000; Goldberg et al., 1981; Lynch and Carroll, 1999; Risner and Goldberg, 1983; Rose and Corrigall, 1997). Compensation for changes in dose by increasing or decreasing intravenous self-administration is less precise for several species of animals, including humans, and results in an inverted U shaped, dose/response curve that is flatter than that obtained for cocaine and other addictive drugs. Similarly, smokers exhibit relatively small and imprecise adjustments in smoking behavior in response to differences in nicotine yield of cigarettes, other than at the extremes (Kozlowski and Herman, 1984; Russell, 1987).

Resistance to extinction is another feature that both smoking in humans and nicotine self-administration in animals have in common (Di Chiara, 2000; Goldberg et al., 1981; Rose and Corrigall, 1997; Shoaib and Stolerman, 1999, and see results presented below). Withdrawal of nicotine, or its pharmacological blockade, results in significant decreases in self-administration but typically not complete cessation of operant responding within the period tested (reviewed in Di Chiara, 2000).

Di Chiara (2000) speculated that in well trained animals that have been chronically exposed to nicotine these two features — poor regulation of self-administration by dose and resistance to extinction — may reflect a shift in the control of self-administration behavior from the consequences of the behavior, i.e., the motivational effects of nicotine, to a more automatic mechanism, whereby stimuli conditioned to the drug trigger motor plans, i.e., self-administration behavior, that are relatively independent of the drug. A similar proposal had been made earlier by Tiffany (1990) for smoking in humans.

Another feature that is difficult to explain by a simple nicotine reinforcement model is the persistence of smoking and self-administration in the face of short-term desensitization and long-term inactivation of nAChRs, and of the acute and chronic tolerance to nicotine that presumably results from these receptor changes. Both smokers and animals who self-administer nicotine are chronically exposed to the drug; regular smokers sustain plasma levels of 20–30 ng/ml of nicotine during the day, with peaks as high as 100 ng/ml (Benowitz et al., 1983; Henningfield et al., 1993). Rats self-administering doses of nicotine that maximize fixed ratio responding achieve plasma levels of approximately 40–120 ng/ml within a single 1–2-h daily session after 20 or more sessions (Donny et al., 2000b; Shoaib and Stolerman, 1999). Acute exposure to this amount of nicotine would be expected to desensitize nAChRs, and more prolonged exposure to upregulate nAChRs, presumably as a reflection of receptor inactivation. In both cases, acute and chronic tolerance, respectively, to the behavioral and physiological effects of nicotine would also be predicted. Indeed, acute tolerance within a session is suggested by the observation that the plasma corticosterone response to the last nicotine infusion of a 1-h self-administration session is diminished, relative to the rise after the first self-administered infusion of that session (Donny et al., 2000a, unpublished data). Moreover, rats that acquired stable nicotine self-administration over 20 1-h daily sessions, exhibited a moderate, linear increase in intake of approximately 15% when tested for an additional 9 days (Donny et al., 2000b), suggesting that a modest amount of chronic tolerance developed to either the reinforcing (Hammer et al., 1997; Tella et al., 1999) or rate-limiting (Schenk and Patridge, 1997) effects of the drug. Increased agonist binding in the brain (Benwell et al., 1988; Breese et al., 1997) and evidence for both acute and chronic tolerance to nicotine (Perkins et al., 2001a, 1994) have been reported for smokers. Acute tolerance to most effects of nicotine in humans generally is not lost for several hours following nicotine exposure (e.g., Perkins et al., 1995), which is much longer than the typical interval between cigarettes (30–60 min, Hatsukami et al., 1988). This observation indicates that, following the first cigarette of the day, most cigarettes are smoked in the presence of maximal acute tolerance to nicotine.

If acute and chronic tolerance develop because nAChRs are in a state of acute desensitization within a smoking day or self-administration session and chronic inactivation across days/sessions, then why does the behavior persist? Several explanations have been proposed. As previously stated, subtypes of nAChRs differ in the rate and completeness of desensitization and in their rate of recovery. It may be that the receptor subtype(s) responsible for nicotine's reinforcing effects show less desensitization relative to those that mediate other effects of nicotine and therefore remain fully functional even after prolonged exposure. There is some evidence for regional and subtype specificity in several of nicotine's behavioral and physiological effects (Iwamoto, 1991; Pagliusi, 1996; Panagis, 1996). However, nAChRs containing the $\alpha 4$ subunit, such as the $\alpha 4\beta 2$ subtype, account for nearly all of the [^{3}H]nicotine binding in the rodent brain (Flores et al., 1992), and have been implicated in many of the behavioral and physiological effects of nicotine (Marubio et al., 1999), most of which would be expected to exhibit tolerance with chronic exposure (Hulihan-Giblin et al., 1989; McCallum et al., 1999; Stolerman et al., 1973). Since the existence of receptors containing the $\beta 2$ subunit is also critical for nicotine self-administration (Epping-Jordan et al., 1999; Picciotto et al., 1998), there is little direct evidence, at present, to support the hypothesis that nicotine reinforcement is fundamentally segregated from all other actions of nicotine by receptor subtype and susceptibility to tolerance. This argument is also made less compelling by the finding that the effectiveness of nicotine in stimulating dopamine release from dopaminergic projections to the shell of the nucleus accumbens, which is thought to be of critical importance for nicotine's reinforcing effects, is readily diminished by continuous

prior infusion of nicotine, suggesting desensitization of the relevant receptors (Balfour et al., 2000). Consistent with this finding are the results of a self-administration study in which nicotine was continuously available. Under these circumstances, self-administration is largely restricted to the dark (active) period of the lighting cycle. Increased DA efflux in the nucleus accumbens, as measured by microdialysis, was associated with infusions at the beginning of each dark period but not with infusions later in that period (Matta, personal communication). It may also be the case that nicotine self-administration is maintained by a balance of active and desensitized upregulated receptors (Dani and Heinemann, 1996). Indeed, there is evidence that some types of nicotinic receptors can be upregulated but still functional (Hsu et al., 1996; Ke et al., 1998; Wang et al., 1998). Depending on nicotine levels, timing and receptor subtype, different nAChRs may be resting, open, desensitized or inactivated. Overnight abstinence in smokers, and the 23-h abstinence typical of limited access self-administration procedures in rats (Donny et al., 2000b), would allow for resensitization of some receptors. The increased number of active receptors might result in increased positive reinforcement early in the session combined with withdrawal symptoms because of cholinergic hyperactivity. Sustained self-administration may thus result from a combination of the heightened positive reinforcement early in the session and the need to reduce withdrawal by desensitizing the overactive cholinergic system. Consistent with this idea are data showing that the first cigarette of the day has enhanced reinforcing effects (Fagerstrom and Schneider, 1989). The possible role of withdrawal in maintaining smoking behavior is further suggested by findings that rats continuously infused with nicotine show behavioral signs of withdrawal (Malin et al., 1992, 1994), and nicotine withdrawal raises the threshold of brain stimulation reward (Epping-Jordan et al., 1998). Comprehensive reviews of animal research on nicotine withdrawal (Malin, 2001, in press), and on its neurobiological bases (Kenny and Markou, 2001, in press), can be found elsewhere in this issue. However, recent research indicates that 1-h daily self-administration sessions do not result in significant increases in nicotine withdrawal symptoms following a mecamylamine challenge 25 h after the last session (Watkins et al., 1999). While more subtle, affective signs of withdrawal cannot be ruled out (Kenny and Markou, 2001, in press), these data suggest that avoidance of withdrawal is not, by itself, entirely sufficient to explain maintenance of self-administration behavior in rats.

Indirect evidence that smoking behavior is influenced by factors in addition to nicotine also comes from the many studies showing the persistence of smoking behavior following pretreatment with novel forms of nicotine administration, such as gum, patch and nasal spray. If smoking behavior was driven exclusively by nicotine intake, then greater nicotine pretreatment should result in a corresponding reduction in smoking behavior, often referred to as "downward compensation" (Perkins et al., 1992). However, no study has shown complete compensation in smoking behavior following nicotine pretreatment, and many show no significant decline at all in smoking behavior. For example, Benowitz et al. (1998) demonstrated that only very high doses of transdermal nicotine could significantly reduce ad lib smoking behavior in smokers not trying to quit. Nonsignificant reductions in daily smoking of only 3% and 10%, respectively, were observed during 5 days each of 21 mg (one patch) and 42 mg transdermal nicotine (two patches) compared to smoking while wearing placebo patches. Thus, 42-mg nicotine pretreatment, which resulted in plasma nicotine concentrations of approximately 40 ng/ml above that typically observed in most smokers and therefore sufficient to completely replace nicotine intake from smoking, produced no significant change in smoking behavior. Only 63-mg nicotine (three active patches at once), resulting in average plasma nicotine concentrations above 50 ng/ml or twice the typical levels from ad lib smoking, produced a significant 40% decline in number of cigarettes per day. Although the slow kinetics of nicotine intake from patch may partly explain the minimal effect of this nicotine pretreatment on smoking behavior, somewhat similar results have been observed with rapid methods of nicotine pretreatment, such as nasal spray (Perkins et al., 1992).

4. The importance of associative cues in smoking

We (Donny et al., 1999b, 2000b) and others (Balfour et al., 2000; Di Chiara, 2000; Rose and Corrigall, 1997) have suggested that environmental cues associated with nicotine delivery are capable of maintaining nicotine-seeking behavior even in the absence of a pharmacological effect of the drug. This suggestion is consistent with a large body of literature demonstrating the importance of conditioned cues in self-administration of other drugs of abuse (Arroyo et al., 1998; DeWitt and Stewart, 1981; Goldberg et al., 1981; Markou et al., 1993; Robinson and Berridge, 1993; Schenk and Partridge, 2001, in press; See et al., 1999; Spealman et al., 1999) and with the belief that conditioning to environmental stimuli may play a significant role in the process of drug dependence and relapse in humans (Childress et al., 1992; Margolin and Avants, 1992; O'Brien et al., 1998). According to this view, "only intermittent pharmacological actions of nicotine, during temporary reversals of receptor desensitization, may be required to sustain operant behavior when combined with contingent presentation with drug-related cues. Drug-related cues may then maintain behavior when nicotine itself has little pharmacological effect" (Donny et al., 2000b, p. 401). Balfour et al. (2000, p. 73) go even further to suggest that "...at times when the plasma nicotine concentration is sufficiently high to cause desensi-

tization of the receptors, tobacco smoking is maintained by the conditioned reinforcers present in tobacco smoke. The hypothesis predicts, therefore, that conditioned reinforcement may play a more important role in the addiction to tobacco than for most other addictive behaviors.''

The influence of nonpharmacological features of cigarette smoking on smoking behavior has received relatively little research attention. However, several studies, notably those conducted by Rose and colleagues, have examined the effects of either: (1) providing sensory smoking cues (e.g., sight and smell of smoke, handling of cigarette) in the absence of nicotine or (2) blocking some of these cues while smoking but allowing the delivery of nicotine via inhalation. Butschky et al. (1995) compared subjective responses of briefly abstinent (overnight) smokers to controlled and blinded exposure to denicotinized cigarettes (''Next'' brand) versus a standard nicotine brand (''Marlboro regular''). Compared to the nicotine cigarettes, the denicotinized cigarettes produced an equal increase in expired-air carbon monoxide, showing equal smoke intake, but far less increase in plasma nicotine (+2 ng/ml versus +11 ng/ml following four exposures to each cigarette type). Yet, subjective ratings of ''liking'' and ''satisfaction'' for the denicotinized cigarettes systematically increased with increasing exposure, relative to a nontobacco control cigarette made of lettuce leaves. Moreover, the denicotinized cigarettes significantly reduced subjective craving and withdrawal to the same degree as did smoking the regular nicotine cigarettes. Gross et al. (1997) found similar results. In a review of 10 studies conducted by Rose and colleagues, Brauer et al. (2001) found that smokers who rated denicotinized cigarettes as being closer in ''liking'' and ''satisfaction'' to nicotine cigarettes tended to be those who were more heavily dependent. This finding suggests that nonnicotine characteristics of smoking (i.e., ''cues'') may be more important in maintaining smoking behavior after the establishment of tobacco dependence than in fostering onset of dependence.

Other research shows that similar subjective effects can be obtained without administering smoke cues themselves but from use of devices that mimic some of the sensory stimulation from smoking. For example, use of a citric acid aerosol, which provides some of the throat sensations experienced from smoking, acutely increases liking and satisfaction, although to a smaller degree than smoking nicotine cigarettes (Levin et al., 1990). Similar results have been seen with administration of capsaicin (Behm and Rose, 1994) and black pepper extract (Rose and Behm, 1994) in abstinent smokers. Moreover, citric acid aerosol has been shown to enhance smoking cessation outcome rates due to nicotine patch treatment at 10 weeks postquit, although this beneficial effect does not persist at later follow-up (Westman et al., 1995). Unlike the other research on smoking cues discussed here, these sensory effects may reflect unconditioned as well as conditioned peripheral effects of nicotine, rather than solely nonnicotine stimuli conditioned to the central effects of nicotine via smoking. Nicotine inhalation is known to produce peripheral sensations in the trachea and lungs (Rose and Levin, 1991).

Also, relevant to understanding the influence of smoking cues on smoking reinforcement is the substantial literature showing increases in craving or ''desire to smoke'' when smokers (abstinent or nonabstinent) are presented with smoking cues, such as a lit cigarette resting in an ashtray, but are not allowed to smoke (Perkins, 1994). Recent research suggests that neutral stimuli paired with availability of smoking can come to elicit increases in smoking urges (Lazev et al., 1999), and greater time spent smoking (Mucha et al., 1998) and can evoke physiological responses indicative of their appetitive nature (Geier et al., 2000), more directly demonstrating the development of conditioned incentive stimuli as triggers for smoking. Finally, in a study of the craving induced by nicotine-withdrawal versus smoking-related cues, Tiffany et al. (2000) reported that transdermal nicotine patches attenuated craving and other effects induced by abstinence from smoking, which are presumably direct results of nicotine withdrawal, but had no selective effect on the craving or any other reactions elicited by smoking cues.

Fewer studies have examined the opposite manipulation, blockade of cues but not of nicotine while smoking in order to see a reduction in subjective responses and smoking behavior. Results from the limited research does indicate that such blockade can reduce subjective liking, satisfaction and, perhaps, smoking reinforcement (self-administration). Rose et al. (1985) anesthetized the respiratory airway with lidocaine to block the throat sensations from smoking and found reduced subjective smoking ''satisfaction.'' Similarly, Baldinger et al. (1995) blocked olfactory cues from cigarette smoking with nose clips and showed reduced taste and enjoyment of smoking. More recently, Perkins et al. (2001a) blocked both the visual and olfactory cues from smoking with opaque goggles and nose clips, respectively, and found significantly reduced ratings of liking and satisfaction, as well as attenuated smoking self-administration. In a follow-up study, separate manipulation of visual versus olfactory cues showed that the olfactory cues were more important but primarily in women; men showed a smaller reduction in the subjective and behavioral effects of smoking due to olfactory blockade. Ability to inhale smoke was not impeded by any of these manipulations, indicating that these results were due to the removal of smoking cues and not to an increase in the response requirements (e.g., intensity of puffing required) or to reduced nicotine intake while smoking under these conditions.

In a study particularly relevant to the question of the relative importance of cues versus nicotine, Rose et al. (2000) administered a continuous intravenous infusion, pulsed intravenous nicotine (to simulate the pattern of intake from puffing) or intravenous saline to three different groups of briefly deprived smokers. Subjects received the intravenous treatment on each of 2 days, in which they either

smoked a denicotinized cigarette or did not smoke at all. A fourth group received intravenous saline while smoking their own regular nicotine brand. The denicotinized cigarettes alone (i.e., cues) increased smoking satisfaction to the same degree as smoking a nicotine-containing cigarette, but neither intravenous nicotine treatment influenced satisfaction. However, the reduction in "craving" due to smoking the denicotinized cigarette was greater when combined with intravenous nicotine so that both together produced a reduction in craving equal to that of smoking a regular nicotine-containing cigarette. Thus, cues alone (i.e., denicotinized cigarette) fully increased satisfaction while nicotine alone (intravenous nicotine) had no effect. Yet, both nicotine plus cues were necessary for complete reduction of craving.

The importance of conditioned cues in maintaining smoking may also at least in part explain the relatively modest success of nicotine-based treatments in smoking cessation programs (Balfour and Fagerstrom, 1996). These preparations would not be expected to fully treat smoking behavior if highly addicted people smoke both for the reinforcing and withdrawal suppressing effects of nicotine (when receptors are in an active state) and for the conditioned reinforcers associated with the drug (when receptors are relatively inactive) (Balfour et al., 2000).

5. Evidence that associative cues influence nicotine self-administration in animals

Most studies of nicotine self-administration in animals have employed paradigms in which nicotine infusions are systematically paired with one or more nonpharmacological stimuli (Ator and Griffiths, 1993; Bardo et al., 1999; Corrigall and Coen, 1989; Donny et al., 1995; Goldberg et al., 1981, 1983, 1989; Lynch and Carroll, 1999; Shoaib et al., 1997; Slifer and Blaster, 1985; Stolerman et al., 1999; Tessari et al., 1995; Valentine et al., 1997; Watkins et al., 1999). However, in contrast to the research on other self-administered drugs such as stimulants and opiates (Markou et al., 1993), only a few studies have provided evidence that directly or indirectly bears on the role of such stimuli for nicotine self-administration. Goldberg et al. (1981) found that in squirrel monkeys self-administering nicotine on a second-order schedule of reinforcement, responding for nicotine was reduced by about 50%, by omitting a brief light stimulus that had become associated with the drug. In one animal who continued to respond at high rates after saline was substituted for nicotine, response rates decreased after the light cue was also omitted. These observations led Goldberg and Henningfield (1988, p. 227) to conclude that, "There are several parameters which can function to substantially strengthen the behavior which leads to nicotine ingestion. . .[including]. . .intermittent presentation of nicotine-paired stimuli. . ."

Two studies have reported the reinstatement of previously extinguished nicotine self-administration behavior by noncontingent "priming" injections of nicotine in rats (Chiamulera et al., 1996; Shaham et al., 1997). In the latter study, spontaneous recovery of the previously extinguished behavior was also observed when rats that had been housed for 21 days without being exposed to the drug-taking environment were then re-exposed to that environment. This recovery of bar pressing may have been triggered by the context (operant boxes) previously associated with iv nicotine self-administration (Di Chiara, 2000).

We have recently reported that drug-related cues can help maintain nicotine-seeking behavior in rats (Donny et al., 1999b). Animals self-administered nicotine on a progressive ratio (PR) schedule of reinforcement in which infusions were coupled to the onset of a 1-s cue light and the initiation of a 1-min time-out period during which the chamber light was turned off and responding was recorded but not reinforced. When tested over a 5-day extinction period, during which saline was substituted for nicotine, the continued contingency between these light cues and bar pressing resulted in a significantly higher number of infusions than when the cues were also omitted.

Given the postulated role of cues in maintaining smoking behavior and early evidence from our laboratory that the presence of nicotine-associated lighting events retarded extinction on a PR schedule of reinforcement in rats (Donny et al., 1999b), we set out to systematically determine the extent to which the robust self-administration behavior obtained using this general paradigm is dependent on the nicotine-associated light cues. Because the following studies have not been reported prior to this paper, we will first review the general methodology used in our laboratory before discussing the specific methods and results for each of the studies.

5.1. General methods

The basic self-administration procedures were derived from the original work of Corrigall (Corrigall, 1992; Corrigall and Coen, 1989) and have been previously described in detail elsewhere (Donny et al., 1995, 1998, 1999b, 2000a, 2000b). Briefly, male Sprague–Dawley rats (Harlan Farms), 41–44 days old and weighing 200–225 g, were individually housed on a 12-h reverse light/dark cycle (lights off 7:00 a.m.). Following habituation, animals were implanted with a catheter into the right jugular vein and treated postsurgically with Timetin (antibiotic), heparin and streptokinase (see Donny et al., 2000b for details). Prior to nicotine self-administration, rats were trained to lever-press for food reinforcement in one session. Following food training, animals were fed 20 g shortly after each experimental session for the remainder of the study. This feeding schedule results in the gradual weight gain of approximately 15 g/week (Donny et al., 1995).

Lever training and all subsequent experimental sessions described below took place in a $10 \times 12 \times 11$ in.^{3} operant chamber (BRS/LVE model # RTC-020) with identical

inactive and active levers, a cue light located 2 in. above the active lever, an overhead chamber (house)-light and a food pellet dispenser, located between the two levers. During all self-administration sessions, animals were connected to a drug-delivery swivel system that allowed practically unrestricted movement in the chamber. An interfaced computer software package (Med Associates, MED-PC 2.0) was used to control reinforcement schedules and record active lever responses, inactive lever responses and infusions.

Unless otherwise specified, during each daily session, active lever responses resulted in a 1-s infusion (0.1 ml/kg) of nicotine bitartate (0.03 mg/kg/infusion: Sigma; dose reported as free base). This dose has been found by several investigators to be at or near the peak of the dose/response curve for nicotine self-administration on a limited access, FR schedule (Corrigall, 1992; Corrigall and Coen, 1989; Donny et al., 1995, 2000b). Infusions were coupled to the onset of a 1-s cue light and the initiation of a 1-min time-out period during which the house light was turned off and responding was recorded but not reinforced. These response-contingent changes are referred to throughout this paper as our "normal cue conditions." A constant background noise of approximately 75 dB produced by exhaust fans located within each sound-attenuating chamber masked the auditory cues associated with drug delivery (e.g., infusion pump). Responding on the inactive lever had no consequence for any of the groups.

All statistical analyses used the mean of the last 2 days of the maintenance, extinction and reacquisition phases. This was done because there was an unequal number of sessions for each phase, so some cut-off was necessary to have an equal number of factors in the repeated measures ANOVA, and it was judged that the last 2 days best characterize stable responding at a particular phase. The extinction and reacquisition experiment was analyzed using separate mixed ANOVAs for extinction and reacquisition. Extinction analyses focused on three groups divided according to extinction parameters and two phases (maintenance and extinction). Reacquisition analyses were performed on six groups based on the combination of extinction and reacquisition parameters and two phases (extinction and reacquisition). Analysis of the PR experiment was conducted with a one-way ANOVA using the four phases (nicotine + cues, saline + cues, saline + no cues and saline + cues) to determine changes in the number of infusions earned on the last 2 days at each phase.

5.2. Maintenance, extinction and reacquisition of self-administration behavior on a fixed ratio schedule

In order to determine the role of nicotine-associated cues in the rate of nicotine-seeking behavior, this experiment examined the effect of removing cues, nicotine or both on self-administration, as well as the ability of cues, nicotine or a combination of both, to reinstate self-administration behavior. Trained rats with functional catheters ($n = 46$) were allowed to acquire nicotine (0.03 mg/kg iv) self-administration (SA) in daily 1-h sessions. All infusions were coupled to the presentation of a 1-s cue light, and the initiation of a 1-min time-out period signaled by offset of the house light. An FR1 schedule was employed for the first 5 days, FR2 for days 6–8, and FR5 for the remainder of the experiment (see Fig. 1 for an overview of the design).

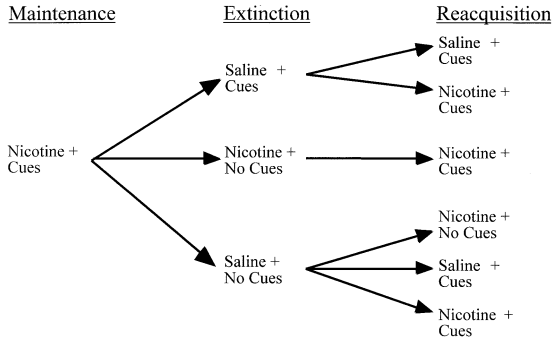

Fig. 1. Experimental conditions and groups during the maintenance, extinction and reacquisition phases of bar pressing on an FR5 schedule. See text for explanation.

5.2.1. Extinction phase

Following the 20-day acquisition period, animals were divided into three groups for a period of 12 days (Extinction Phase). For animals in the saline + cues group ($n = 17$), nicotine was replaced with saline, but the cues (house light and cue light) that had been associated with nicotine delivery throughout acquisition continued to be presented contingent on the animals' behavior. Rats in the nicotine + no cues group ($n = 8$) continued to receive nicotine upon completion of the response requirement, but all infusions were delivered without the simultaneous presentation of cues. Finally, animals in the saline + no cues group ($n = 21$) responded for contingent infusions of saline in the absence of cues. All extinction sessions were carried out on an FR5 schedule of reinforcement.

5.2.2. Reacquisition phase

Subsequent to assessing the rate and degree of extinction, we further examined the role of nicotine-associated cues in self-administration by further subdividing the three extinction groups and replacing the nicotine, the cues or both. For the reacquisition period, all animals were allowed to self-administer on an FR5 schedule for an additional 5 days. During this period (reacquisition), 9 of the 17 rats that had saline + cues during extinction were given access to nicotine in addition to cues (nicotine + cues), while the remaining 8 animals continued on saline replacement (saline + cues). Rats in the nicotine + no cues group during extinction had cues added back, in addition to nicotine, during reacquisition. Lastly, animals in the saline + no cues extinction group reacquired with either cues alone (saline + cues; $n = 7$),

nicotine alone (nicotine + no cues; $n = 7$) or both nicotine and cues (nicotine + cues; $n = 7$) upon completion of the response requirement. At the beginning of each reacquisition session, all rats received one noncontingent "priming" exposure to the stimulus condition appropriate to their group's designation. Thus, rats in the nicotine + no cues condition received one infusion of nicotine at the start of the session during reinstatement, whereas those in the saline + cues received an infusion of saline accompanied by the 1-s cue light and followed by the offset of the house light for 1 min.

5.2.3. Results

As we have previously reported for our normal cued self-administration paradigm (Donny et al., 2000b), rats that were given access to nicotine paired with both lighting events readily acquired stable and robust self-administration over a 20-day period. When saline was then substituted for nicotine in the saline + cues group ($n = 17$), but the visual stimuli previously associated with nicotine continued to be presented, infusion rates dropped by 58% (Fig. 2A). Interestingly, infusion rates remained stable at this reduced level for the next 12 days. Stabilization of infusion rates

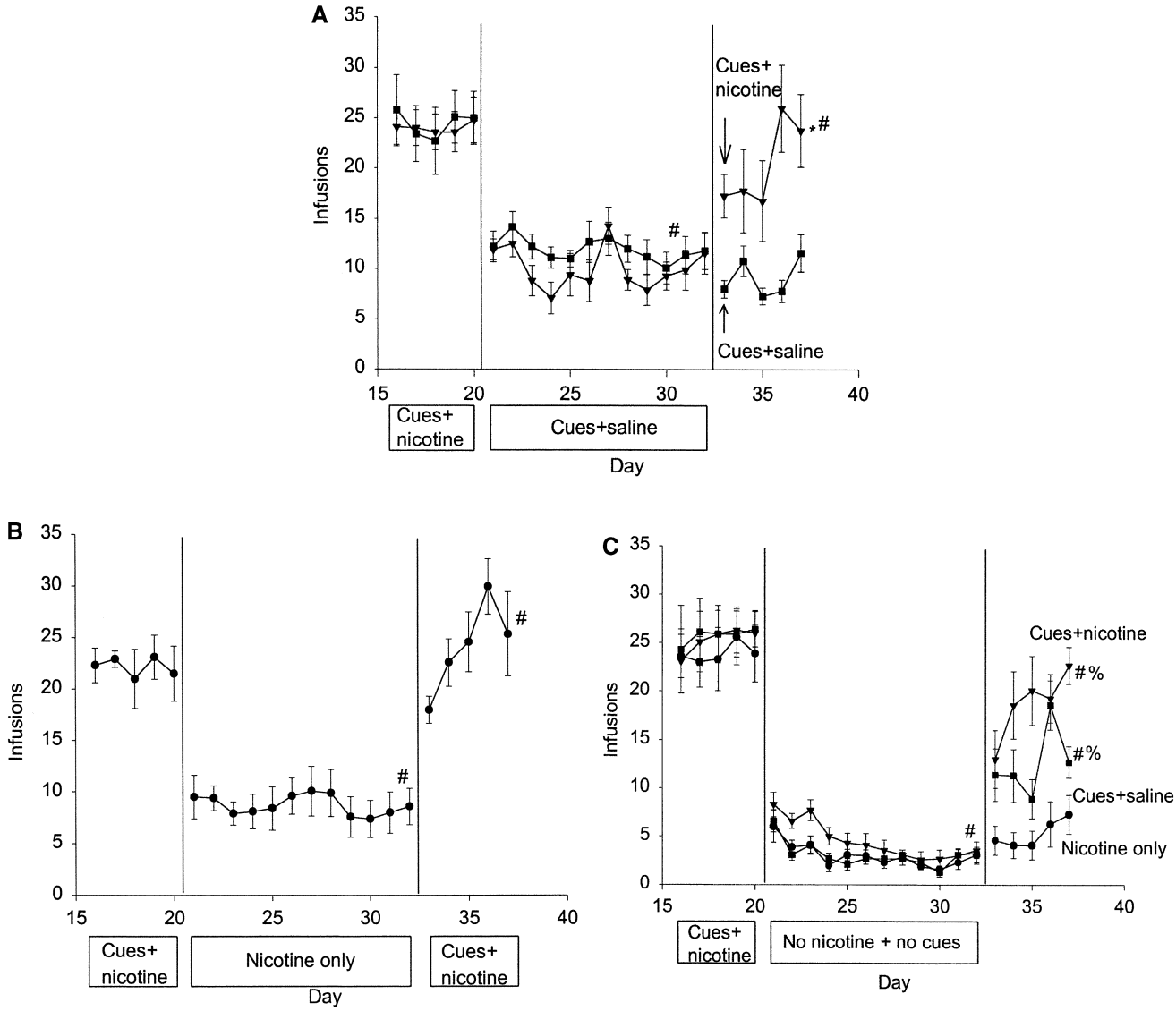

Fig. 2. Mean (±S.E.) number of infusions on an FR5 of rats trained to self-administer nicotine under our normal cue conditions and then either withdrawn from only nicotine with or without subsequent nicotine replacement (2A), withdrawn from only cues with subsequent cue replacement (2B), or withdrawn from both nicotine and cues with subsequent replacement of nicotine, cues or both (2C). In all cases, replacement included priming. $N = 7–9$/group. ANOVA revealed the following significant main and interaction effects: Extinction–Group [$F(2,43) = 3.66$, $P < .05$], Phase [$F(1,43) = 291.85$, $P < .001$], Group × Phase interaction [$F(2,43) = 12.41$, $P < .001$]; Reacquisition–Group [$F(5,40) = 11.09$, $P < .001$], Phase [$F(1,40) = 135.90$, $P < .001$], Group × Phase interaction [$F(5,40) = 14.31$, $P < .001$]. # Indicates a significant within-subject change from preceding phase ($P < .05$). * Indicates a significant difference from the cue only condition in the change from preceding phase ($P < .05$). % Indicates a significant difference from the nicotine only condition in the change from preceding phase ($P < .05$).

well above those seen in a subsequent group that had neither cues nor nicotine (see Fig. 2C; $P < .001$) indicated that the contingent presentation of cues previously associated with nicotine was sufficient to maintain active lever responding. The contingency relationship between these stimuli and the rat's behavior, or their association with nicotine, were not directly manipulated in this study. However, other experiments from this laboratory on the acquisition of nicotine self-administration have established that the effectiveness of these cues is dependent on their being contingent on the animal's behavior, in the presence of nicotine stimulation. Moreover, while one of the stimuli, turning off the house light, appears to have intrinsic reinforcing properties, its association with nicotine is a critical factor in the degree to which it enhances self-administration behavior (Caggiula et al., 2001). When nicotine was reintroduced for nine of the animals in the saline + cues extinction group, the rate of infusions returned to original levels. The other eight rats that remained in the saline + cues condition maintained the ~50% of baseline level of infusions for an additional 5 days (Fig. 2A).

Replacing nicotine with saline and then later adding the nicotine back represents the classic extinction/reinstatement manipulation. As has been reported by several laboratories (see Di Chiara, 2000) including our own (Donny et al., 1995), this procedure results in a drop in response and infusion rates followed by a return to baseline when nicotine is again made available. This is typically considered strong evidence that nicotine is acting as a reinforcer (Goldberg and Henningfield, 1988). However, what is striking about the present data, but has not been thoroughly discussed in the literature before, is the duration for which the saline + cues group continued to demonstrate substantial response rates in the absence of nicotine. After 17 days of saline substitution, we found only partial extinction in the animals still receiving contingent presentations of the cues previously associated with nicotine. Clearly, these lighting cues are capable of maintaining operant responding for an extended period of time at a level above that observed for the same stimuli that had not been paired to nicotine (Caggiula et al., 2001). The ability of nicotine-associated cues to maintain stable responding for at least 17 days contrasts with reports that responding for cocaine-related cues wanes after approximately 5 days (Arroyo et al., 1998). While comparisons across experiments and laboratories are problematic, these data recall the hypothesis, proposed by Balfour et al. (2000) that drug-related stimuli may be more important in sustaining nicotine self-administration and smoking than is the case for other drugs.

Interestingly, the magnitude of extinction that was observed following the removal of nicotine was similar to that in the nicotine + no cues condition ($n = 8$). When cues were withheld, infusion rates dropped by 63% and stabilized at a similar level for the same 12-day period (Fig. 2B); reintroducing the cues reinstated the high rate at which animals earned nicotine infusions almost immediately. Thus, withdrawing and then reintroducing cues associated with nicotine has at least as powerful an effect on self-administration behavior as does withdrawing and replacing nicotine. These changes do not appear to be a result of short-term disruption of behavior resulting from changes in stimulus conditions, since these animals showed no signs of recovering to baseline within the 12-day period. Instead, this downward shift may reflect the postacquisition rate of reinforcement supported by nicotine in the absence of cues.

Finally, we assessed the effects of withdrawing both the cues and nicotine (saline + no cues) and subsequently replacing nicotine (nicotine + no cues), the cues (saline + cues) or both (nicotine + cues). As can be seen in Fig. 2C, when both nicotine and the cues associated with nicotine delivery were removed, infusion rates dropped precipitously to near complete extinction (though response rates remained slightly but significantly higher than on the inactive lever; data not shown). As mentioned above, these data are in stark contrast to the resistance to extinction exhibited when only nicotine was omitted (saline + cues group) and extend a literature demonstrating how cues, even in the absence of nicotine, can maintain self-administration in animals (Di Chiara, 2000; Goldberg et al., 1981) and smoking in humans (Di Chiara, 2000; Rose and Corrigall, 1997). Somewhat surprisingly, when nicotine was again made available, but by itself, following extinction, there was a trend for infusion rates to increase above extinction, but they did not achieve statistical significance over the time period tested ($P > .05$; Fig. 2C). Remarkably, replacing the cues previously associated with nicotine, but not the nicotine, significantly elevated infusion rates above both extinction levels and the level of reacquisition induced by nicotine alone (Fig. 2C). These results suggest that the cues associated with nicotine, even in the absence of nicotine delivery, engender greater self-administration behavior than nicotine itself. This finding that nicotine-associated stimuli play an important role in the reinstatement of operant responding is consistent with a previous report that re-exposing rats to the chamber within which nicotine was self-administered resulted in recovery of bar pressing after 21 drug-free days (Shaham et al., 1997). Finally, as expected, when both cues and nicotine were reintroduced, infusion rates recovered to pre-extinction levels within 5 days (Fig. 2C). Clearly, the combination of nicotine and nicotine-associated cues had a greater effect on reacquisition of responding than nicotine alone.

5.3. Nicotine self-administration on a PR schedule

The experiments presented above demonstrate the importance of environmental cues in the maintenance and reacquisition of nicotine self-administration on a fixed ratio schedule of reinforcement. We have also recently collected some additional data using a PR schedule to supplement our earlier finding using this schedule (Donny et al., 1999b). In a PR schedule, the number of responses required to earn a

single infusion increases with each infusion. By determining how hard each animal is willing to work for the infusions, the PR schedule is better able to separate reward strength from possible satiating effects of cumulative drug doses (Stafford et al., 1998). Self-administration on a PR may be a better measure of incentive salience or craving than the FR schedule (Arnold and Roberts, 1997; Markou et al., 1993; Mendrek et al., 1998). In the present experiment, we evaluated the mean break point (final ratio completed with a 4-h session) when completion of each response requirement was reinforced with either nicotine in combination with the normal cue conditions (nicotine + cues), the nicotine-associated cues paired with saline (saline + cues) or saline without nicotine-associated cues (saline + no cues).

Eight rats acquired nicotine self-administration over a 20-day period using our normal cued FR procedure as described above. They were then switched to a PR schedule where response requirements were increased according to the formula $5 \times \mathrm{EXP}(0.2 \times \text{infusions number}) - 5$ (Depoortere et al., 1993). This schedule results in the following sequence of required responses per infusion: 3, 6, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 179, 219, 268, 328, 402, 492. All PR sessions lasted 4 h. After 5 days on the PR schedule, extinction was initiated by replacing nicotine with saline while continuing the contingent delivery of nicotine-associated cues.

As was seen with the FR schedule, substituting saline for nicotine, while retaining the cue contingency, had only a partial effect on self-administration behavior, reducing the number of infusions earned to approximately 70% of baseline (Fig. 3). This level of responding remained stable over the next 15 days. The cues were then also removed, resulting in only saline infusions upon completion of the response requirement. When saline was delivered without cues, the number of infusions earned on a PR dropped to approximately 40% of baseline within 3–5 days. Finally, replacing the cues but continuing saline returned the number of infusions earned per session to 77% of baseline. The response-contingent delivery of cues previously associated with nicotine is capable of maintaining operant responding on a PR schedule of reinforcement for an extended period of time even in the absence of nicotine delivery.

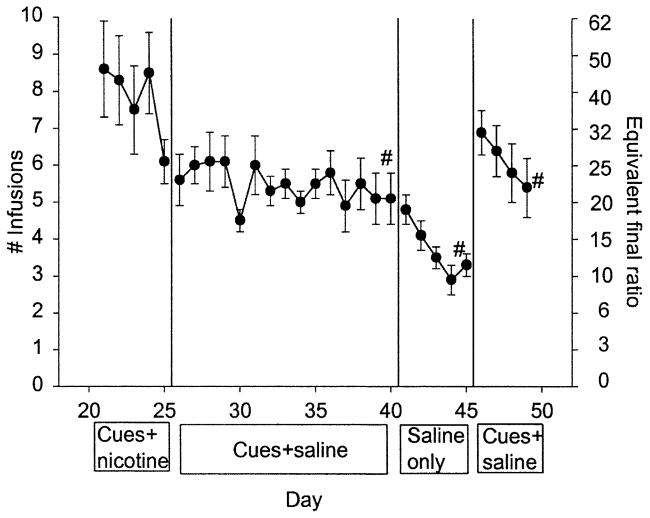

Fig. 3. Mean (±S.E.) number of infusions on a PR of rats trained on nicotine and cues, then withdrawn from nicotine (days 26–40), then withdrawn from both nicotine and cues (days 41–45), with subsequent replacement only of cues (days 46–49). $N = 8$. ANOVA revealed a significant main effect of Phase [$F(3,21) = 19.63$, $P < .001$]. # Indicates significant change from preceding phase ($P < .05$).

6. Conclusions and significance

The present results strongly indicate that, in addition to its intrinsic reinforcing properties, nicotine promotes self-administration behavior by establishing or magnifying the reinforcing effects of associated, nonpharmacological stimuli, such as the visual and/or auditory cues typically found in animal self-administration paradigms. In the absence of nicotine stimulation, these cues are capable of maintaining significant levels of operant behavior for extended periods of time and of reinstating the behavior after extinction. Our findings thus provide strong support for the hypothesis proposed by several investigators that conditioned cues are an important component of nicotine self-administration in animals and smoking in humans (Balfour et al., 2000; Caggiula et al., 2000; Di Chiara, 2000; Donny et al., 1999b, 2000b; Goldberg and Henningfield, 1988; Goldberg et al., 1981; Perkins et al., 2001a; Rose and Levin, 1991; Rose and Corrigall, 1997).

These finding may also explain, at least in part, some anomalous features of smoking and nicotine self-administration that were outlined earlier. For example, the relative insensitivity of smoking and nicotine self-administration to dose would be predicted if dose of nicotine is manipulated against an invariant background of environmental stimuli that are supporting the behavior. Similarly, the tenacity of these behaviors in the face of reduced nicotine stimulation, caused by either absence of the drug or periodic reduction in the sensitivity of the underlying receptor systems, induced by continuous nicotine exposure, can also be explained by the resistance to extinction engendered by continued exposure to conditioned cues alone. Finally, the observations discussed earlier, that smoking is only partly attenuated by nicotine pretreatment, and that nicotine-based treatment strategies have had only limited success, can also be understood within the context of an overdetermined behavior in which stimuli conditioned to the drug trigger motor plans, i.e., self-administration behavior (Di Chiara, 2000) or smoking (Tiffany, 1990), that are relatively independent of its pharmacological consequences, i.e., nicotine stimulation.

These results may have implications for research on smoking behavior in humans, including treatment for smoking cessation. The contribution of smoking cues to

maintaining cigarette smoking and to relapse has not been the subject of much systematic investigation, despite the studies noted previously. Thus, human research should focus more specifically on the influence of these cues, conditions in which their influence is enhanced or reduced, and individual differences in the magnitude of their impact on smoking maintenance (e.g., Perkins et al., 2001a). In terms of interventions for smoking cessation, these results may lend support to the notion that overreliance on nicotine replacement alone as the standard of treatment is inadequate (Shiffman, 1993). Nicotine replacement treatment may aid in relieving specific nicotine withdrawal symptoms but is unlikely to have any influence on conditioned effects of smoking cues (Tiffany et al., 2000), which likely requires behavioral counseling. One component of formal behavioral counseling aimed at helping smokers quit involves teaching them to avoid or otherwise cope with cues associated with smoking (Shiffman and Cline, 1990), such as by avoiding smoking sections of restaurants or by asking friends not to smoke in front of them. However, this component of counseling is often omitted or presented too briefly in treatment provided in most health care settings, in which counseling (if provided at all) is limited to only 5–10 min of suggestions. Based on the importance of cues to maintaining smoking behavior, as outlined in this review, counseling should increase its emphasis on reducing the impact of these cues, by increasing time spent in aiding coping strategies and by developing more powerful interventions to prevent conditioned reinforcing effects of cues, perhaps even through medications (Hutchison et al., 1999).

Finally, the possibility that nicotine-paired cues may be as important as nicotine in maintaining smoking behavior and nicotine self-administration should refocus our attention on the neurobiology of conditioned reinforcers. As previously stated, nicotine, like other drugs of abuse, increases the release of dopamine from terminal fields of the mesolimbic dopamine system (Balfour et al., 2000; Di Chiara, 2000), and activation of this system has been implicated in the reinforcing and addictive properties of abused drugs (Koob et al., 1998; Robinson and Berridge, 1993; Wise and Bozarth, 1987), including nicotine (Balfour et al., 2000; Di Chiara, 2000). However, there is a growing body of evidence that drug-induced release of dopamine may be more important for earlier stages in the addictive process. Other patterns of neuronal activity may become more important as the process proceeds, depending on several factors, including, but not limited to the prior drug experience of the animal, whether observations are made during drug-seeking or drug-taking, and whether the response being measured is to the drug or to drug-related cues (Carelli and Ijames, 2000; Chang et al., 2000; Ito et al., 2000). For example, exposure of both drug-experienced laboratory animals and humans to drug-related cues alone can activate several structures linked to mesocorticolimbic circuitry implicated in the formation of associations between addictive drugs and environmental stimuli (Childress et al., 1999; Schroeder et al., 2000; Sell et al., 2000). When rats were tested for cocaine-or cue-induced reinstatement of operant responding after extinction to cocaine, reversible bilateral inactivation of the nucleus accumbens attenuated lever pressing for cocaine but not for cocaine-related cues, whereas inactivation of the basolateral amygdala had the opposite effect: it reduced responding for cues but not for cocaine (Grimm and See, 2000). These and other studies suggest that the search for mechanisms that underlie smoking should be broadened beyond those related to the direct neurobiological actions of nicotine and include the associative processes by which nicotine promotes the establishment or magnifies the salience of conditioned reinforcers.

Acknowledgments

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