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Repeated Nicotine Exposure Enhances Reward-Related Learning in the Rat

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Repeated exposure to addictive drugs causes neuroadaptive changes in cortico-limbic-striatal circuits that may underlie alterations in incentive-motivational processes and reward-related learning. Such drug-induced alterations may be relevant to drug addiction because enhanced incentive motivation and increased control over behavior by drug-associated stimuli may contribute to aspects of compulsive drug-seeking and drug-taking behaviors. This study investigated the consequences of repeated nicotine treatment on the acquisition and performance of Pavlovian discriminative approach behavior, a measure of reward-related learning, in male rats. Water-restricted rats were trained to associate a compound conditioned stimulus (tone+light) with the availability of water (the unconditioned stimulus) in 15 consecutive daily sessions. In separate experiments, rats were repeatedly treated with nicotine (0.35 mg/kg, s.c.) either (1) prior to the onset of training, (2) after each daily training session was completed (ie postsession injections), or (3) received nicotine both before the onset of training as well as after each daily training session. In this study, all nicotine treatment schedules increased Pavlovian discriminative approach behavior and, thus, prior repeated exposure to nicotine, repeated postsession nicotine injections, or both, facilitated reward-related learning.

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INTRODUCTION

Repeated exposure to drugs of abuse produces long-lasting behavioral alterations and neural adaptations in cortical, limbic, and striatal brain regions (Robinson and Berridge, 1993, 2000; O'Brien *et al*, 1998; Jentsch and Taylor, 1999; Berke and Hyman, 2000; Hyman and Malenka, 2001; Vanderschuren and Kalivas, 2000; Nestler, 2001). These cellular and physiological changes have been argued to occur within neuronal systems involved in incentive motivation, behavioral control, and learning and memory processes. We have hypothesized that drug-induced changes in cortico-limbic-striatal brain circuits result in enhanced incentive-motivational salience of drugs and drug-associated stimuli as well as deficits in inhibitory control processes (Jentsch and Taylor, 1999, 2000; Olausson *et al*, 2000, 2002; Taylor and Jentsch, 2001). These drug-induced alterations could lead to neurocognitive deficits (Rogers and Robbins, 2001) and, together, such motivational-cognitive alterations may contribute to the expression of addictive behaviors and compulsive drug-seeking and –taking behaviors.

The compulsive use of drugs observed in smokers and drug abusers is commonly associated with stimulus-dependent drug-seeking and drug-taking behaviors (cf Tiffany and Carter, 1998; Caggiula *et al*, 2001). Indeed, these behaviors can be influenced by reward-associated conditioned stimuli (CS), or 'cues', that gain the ability to modulate incentive-motivational processes through their acquired conditioned reinforcing qualities. In humans, drug-associated CS precipitate drug craving (O'Brien *et al*, 1998; Childress *et al*, 1999) and produce region-specific activation of cortico-limbic-striatal brain regions (Grant *et al*, 1996; Childress *et al*, 1999; Volkow and Fowler, 2000; Due *et al*, 2002). In rats, reward-associated stimuli support nicotine self-administration (Caggiula *et al*, 2001), produce reinstatement of drug-seeking behavior (Grimm *et al*, 2001; See, 2002), and elicit reward-motivated behaviors, including Pavlovian discriminative approach behavior (Everitt *et al*, 1999; Cardinal *et al*, 2002). The acquisition of such Pavlovian discriminative approach behavior may be a direct measure of processes involved in the ability of reward-associated CS to control behavior. In this task, animals learn to associate a CS with delivery of an appetitive reinforcer. On consecutive days, animals are trained to approach the magazine (ie where the reinforcement is delivered) only during presentation of the CS, or the US. Thus, the subjects learn to discriminate CS and US periods from other times and come to selectively approach the magazine during presentation of the CS or the US. This type of stimulus–

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reward learning may be one functionally relevant mechanism by which drug-associated CS, or cues, support addictive behaviors (Everitt *et al*, 2001).

Psychostimulant drugs can facilitate the acquisition of Pavlovian approach behavior either after single dose treatment (Hitchcott *et al*, 1997) or repeated pre-exposure (Harmer and Phillips, 1998; Taylor and Jentsch, 2001). Nicotine can also facilitate learning and cognitive processes in several different animal models (Rezvani and Levin, 2001), and nicotine has been argued to be particularly effective in establishing or enhancing the incentive-motivational properties of reward-associated conditioned cues (Balfour *et al*, 2000; Caggiula *et al*, 2001). External and sensory conditioned cues may play a critical role in tobacco addiction (Balfour *et al*, 2000; Caggiula *et al*, 2001) and, more specifically, in the maintenance of nicotine-taking behavior (Brauer *et al*, 2001). Consequently, stimuli associated with smoking or nicotine produce craving and support smoking behavior in humans (Mucha *et al*, 1999; Dols *et al*, 2000). Similarly, preclinical experiments have also indicated an important role of nicotine-associated CS in nicotine-seeking and nicotine-taking behavior, and this relation has been demonstrated in the nicotine self-administration paradigm where such stimuli support instrumental behavior even in the absence of nicotine (Caggiula *et al*, 2001). Together, these observations implicate nicotine-induced alterations in cue-elicited behaviors in incentive-motivational processes that are relevant to clinical aspects of smoking and drug addiction. However, no preclinical experiments have directly tested the hypothesis that repeated nicotine exposures facilitate reward-related learning relevant to addiction.

The present study examined the effects of repeated nicotine administration on reward-related learning measured by Pavlovian discriminative approach behavior. Specifically, we investigated whether repeated daily nicotine treatment for 15 days administered prior to the onset of behavioral testing would enhance Pavlovian discriminative approach behavior. In addition, nicotine's effect on Pavlovian discriminative approach behavior was further examined by giving animals repeated nicotine injections administered 5 min after the completion of each of 15 daily training sessions (ie 'postsession' treatment). This treatment strategy can be used to dissociate the acute effects of pharmacological manipulations on learning and memory processes from effects of task performance and competing behaviors (McGaugh, 1966). We further evaluated the potentially synergistic effects of a combination of repeated nicotine pretreatment prior to learning and daily postsession nicotine injections. Together, these studies are critical in determining how repeated nicotine administration affects processes implicated in the control of behavior by reward-related learning that may have relevance to nicotine addiction.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats ($n = 76$) weighing 250–300 g at the start of the experiments, were supplied by Charles River (Portage, ME, USA). The rats were housed in pairs under constant cage temperature (20–21°C), humidity (40–50%), and a controlled 12/12 h light–dark cycle (light on at 7 am and off at 7 pm), and were initially allowed 7 days to adjust to the housing facilities. The rats had free access to food at all times. Water was available *ad libitum* until 3 days prior to the first day of training, and immediately after the 15-day training phase was completed. During the 3 days prior to the start of training, animals were restricted to 30 min access to water per day. During the testing period, water was intermittently available in the operant chambers according to the behavioral task protocol (see below) as well as in the home cage for 30 min, beginning 30 min after the daily testing session. The experiments in the present study were approved by the Yale University Animal Care and Use Committee (YACUC) and followed the NIH *Guide for the Care and Use of Laboratory Animals*.

Drugs

(−)-Nicotine ditartrate (Sigma, USA) was dissolved in a sterile 0.9% sodium chloride solution, and the pH of the nicotine solution was neutralized with sodium bicarbonate. Nicotine was injected subcutaneously (s.c.) at 2 ml/kg. The dose of nicotine is expressed as the weight of the free base of nicotine.

Experimental Techniques

Locomotor activity. Locomotor activity was measured using automated activity meters (Digiscan animal activity monitor, Omnitech Electronics, USA). The activity meters were equipped with two parallel rows of infrared photosensors, each row consisting of 16 sensors placed 2.5 cm apart. The activity meters were controlled by and data from the activity meters collected by a PC using the Micropro software (Omnitech Electronics, USA).

Rats were placed in transparent plastic boxes that were fitted into the activity meters. The rats were initially allowed to habituate to the locomotor activity recording equipment for 30 min, after which they were taken out, injected with nicotine or vehicle, and placed back into the boxes. Locomotor activity was then recorded for 60 min starting 5 min after drug injection. All experiments were performed between 8 am and 6 pm.

Pavlovian discriminative approach behavior

Schedule using paired presentation of the CS and the US: Standard aluminum operant chambers ($30 \times 20 \times 25\,cm^3$) with grid floors (Med Associates Inc., USA) were used to study the acquisition of Pavlovian discriminative approach behavior. Each operant chamber was housed in a sound attenuating outer box equipped with a white noise generator and a fan to reduce external noise. A liquid dipper (0.06 ml) delivered water as the reinforcer into the magazine. Head entries were detected by a photocell mounted within the magazine, above the reinforcer receptacle. Above the magazine was a 2.5 W, 24 V light. The operant chamber was illuminated by house light mounted on the back wall. A Sonalert tone (10 kHz) generator was mounted above the magazine. A PC with interface and the MedPC software (Med Associates Inc., USA) controlled the boxes.

On the first day, 5-s access to 0.06 ml water (the unconditioned stimulus (US)) was available in the dipper on a fixed time 15-s (FT-15) schedule; the session ended after the delivery of 100 reinforcers. Beginning on the second day, the subjects received 30 pairings of a 5-s compound conditioned stimulus (CS; light + tone) followed immediately by 5-s access to 0.06 ml of water; the CS + US pairings were delivered on a random time 30-s (RT-30) schedule. Head entries during the RT-30 interval resulted in a 3-s delay during which time no reinforcement was given, and the RT-30 schedule was restarted. Training on this schedule results in a discriminated pattern of approach of the magazine during CS + US, but not during inter-CS + US, periods.

Schedule using nonpaired presentation of the CS and the US: On the first day, 5-s access to 0.06 ml water (ie the US) was available in the receptacle on a FT-15 schedule; the session ended after the delivery of 100 reinforcers. Beginning on the second day, the subjects randomly received 30 presentations of each of the 5-s CS and 5-s access to 0.06 ml of water. Two independent RT-30 schedules separately controlled delivery of the CS or US; however, the following additional conditions applied: (1) the presentations of the CS and the US never overlapped and were not presented within 5-s of each other and (2) head entries during either of the RT-30 intervals resulted in a 3-s delay during which time no CS or reinforcement was presented, and the RT-30 schedules were restarted.

Experimental Design

Experiment using paired presentation of the CS and the US. Animals ($n = 56$) were randomly divided into two experimental groups. One group ($n = 28$) received daily injections (15 consecutive days) with nicotine (0.35 mg/kg s.c.) and the control group ($n = 28$) received the equal volume of a sterile 0.9% sodium chloride solution. Locomotor activity was recorded on treatment days 1 and 15. These animals were then divided into four balanced groups ($n = 14$), two nicotine-treated and two vehicle-treated. After 3 days of withdrawal, all rats were subjected to the Pavlovian discriminatory approach behavior task described above for 15 consecutive days. During the behavioral testing phase, the animals received either daily injections of nicotine (0.35 mg/kg s.c.) and/or 0.9% sodium chloride 5 min after each training session had been completed, yielding the following four experimental groups (pretreatment/postsession treatment): vehicle/vehicle (Veh/Veh), vehicle/nicotine (Veh/Nic), nicotine/vehicle (Nic/Veh), and nicotine/nicotine (Nic/Nic).

Experiment using nonpaired presentation of the CS and the US. Animals ($n = 20$) were randomly divided into two experimental groups. One group ($n = 10$) received daily injections (15 consecutive days) with nicotine (0.35 mg/kg. s.c.) and the control group ($n = 10$) received an equal volume of 0.9% NaCl. After 3 days of withdrawal, all rats were subjected to the task in which the tone + light CS never predicted US availability, as described above, for 15 consecutive days.

Statistics

The data from the present experiments were evaluated using a one or two-way analysis of variance (ANOVA), for repeated measures where appropriate. In the analysis of locomotor activity, treatment (nicotine or vehicle) was the between-subject factor and treatment day (ie testing on day 1 or 15 of treatment) was used as the within-subject factor. In the ANOVA analyses of reward-related learning, pretreatment (ie treatment prior to onset of behavioral testing; nicotine or vehicle), postsession treatment (ie treatment administered after completion of each daily training session; nicotine or vehicle were there between subject factors. Within-subject measure was training days. *Post hoc* comparisons were performed using Fischer's PLSD, paired *t*-test, Bonferroni–Dunn or Tukey–Kramer's *post hoc* tests where appropriate. A probability value (p) equal to or less than 0.05 was considered statistically significant.

RESULTS

Locomotor Activity Studies

In the locomotor activity experiments, there were two experimental groups, one nicotine-treated and one saline-treated control group. In this experiment, there were statistically significant main effects of treatment ($F_{1,54} = 44.662$; $p < 0.001$) and treatment days (the repeated measure; $F_{1,54} = 19.487$; $p < 0.001$) as well as a significant treatment × treatment day interaction ($F_{1,54} = 32.704$; $p < 0.001$; Figure 1). The *post hoc* analysis, made with Fisher's PLSD test on the data obtained on day 1, demonstrated that acute treatment with nicotine (0.35 mg/kg s.c.) stimulated locomotor activity ($p < 0.001$). A paired *t*-test revealed that daily treatment with nicotine enhanced the behavioral response to a subsequent injection of nicotine ($p < 0.001$). Daily vehicle injections for 15 days did not alter the baseline locomotor activity. Moreover, there were no treatment differences in spontaneous

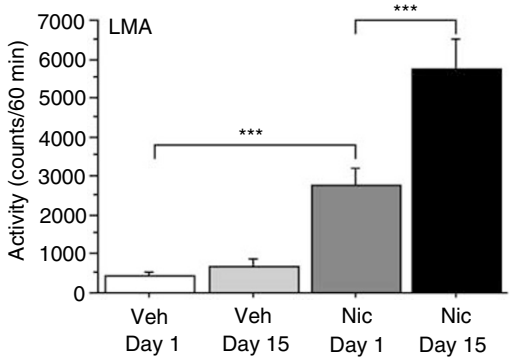

Figure 1 Animals received daily injections with nicotine (0.35 mg/kg, s.c.; $n = 28$) or vehicle ($n = 28$) for 15 consecutive days, and the effect of vehicle or nicotine on locomotor activity was evaluated on treatment days 1 and 15. Acute administration of nicotine (0.35 mg/kg, s.c.; −5 min) increased locomotor activity, and this effect was enhanced on day 15 after repeated nicotine treatment. Repeated treatment with vehicle had no significant effect. Data are presented as the mean total locomotor activity counts/60 min (± SEM). ***$p \leqslant 0.001$ (ANOVA followed by the paired *t*-test).

locomotor activity during the habituation phase on day 15 (data not shown).

Reward-Related Learning

Repeated treatment with nicotine did not alter magazine approach during the initial magazine training session (data not shown). Thus, the repeated drug treatment does not appear to affect primary motivation for water or ability to obtain water from the dipper. We first performed an overall ANOVA on approach during the CS period to probe the main effects of pretraining treatment ('pretreatment') and postsession treatment across days of training (the repeated measure). There were statistically significant main effects of training days (indicating that CS-evoked magazine approach progressively increased as a function of training; $F_{4,728} = 91.393$; $p < 0.0001$), pretreatment ($F_{1,52} = 4.042$; $p < 0.05$) as well as postsession treatment ($F_{1,52} = 4.117$; $p < 0.05$) on magazine entries made during CS presentation (Figure 2a). There were no differences on measures of magazine approach on training day 1 according to Bonferroni–Dunn *post hoc* test, indicating that repeated nicotine treatment did not alter the initial level of performance on this task. Tukey–Kramer's *post hoc* analysis revealed that all nicotine treatment schedules (ie Veh/Nic; Nic/Veh; Nic/Nic) increased CS magazine approach during the behavioral testing phase compared to vehicle-treated control animals.

The Tukey–Kramer test also showed that the CS magazine approach on training day 4 did not significantly differ from that observed on training day 3. Thus, the acquisition of the Pavlovian discriminative approach behavior appeared to occur within the three initial days of training. When analyzed using a repeated measures ANOVA only over these first 3 days of training, there were significant main effects of treatment ($F_{3,52} = 5.908$; $p < 0.05$) and training days ($F_{2,104} = 154.042$; $p < 0.001$), as well as a significant treatment × training day interaction ($F_{6,104} = 2.538$; $p < 0.05$, Figure 3a). Tukey–Kramer's *post hoc* analysis revealed differences in all nicotine-treated groups (ie Veh/Nic; Nic/Veh; Nic/Nic) during the acquisition phase. Together, these results indicate that the nicotine treatment facilitated learning about the stimulus–reward associations.

There was a significant main effect of training days ($F_{4,728} = 125.032$; $p < 0.0001$) and pretreatment on magazine approach during US presentation ($F_{1,52} = 6.213$; $p < 0.05$, Figure 2b); repeated treatment with nicotine prior to training thus resulted in an increased approach to the magazine during US presentation. The Tukey–Kramer *post hoc* test confirmed that this significant difference was attributed to enhanced US approach in animals receiving repeated nicotine treatment before the onset of behavioral testing in combination with postsession nicotine injections (Figure 2b). Nonspecific approach to the magazine during the inter-CS + US period was not affected by nicotine pretreatment ($F_{1,52} = 0.4$; NS) or by postsession treatment ($F_{1,52} = 0.301$; NS, Figure 2c).

In the experiment in which the CS and US were never paired (see Experiment using nonpaired presentation of the CS and the US), prior repeated nicotine treatment did not alter magazine approach during CS ($F_{1,18} = 2.722$; NS, Figure 4a) or US ($F_{1,18} = 0.231$; NS, Figure 4b) periods. On the other hand, prior repeated nicotine treatment increased magazine entries during inter-CS + US periods ($F_{1,18} = 9.427$; $p < 0.001$, Figure 4c).

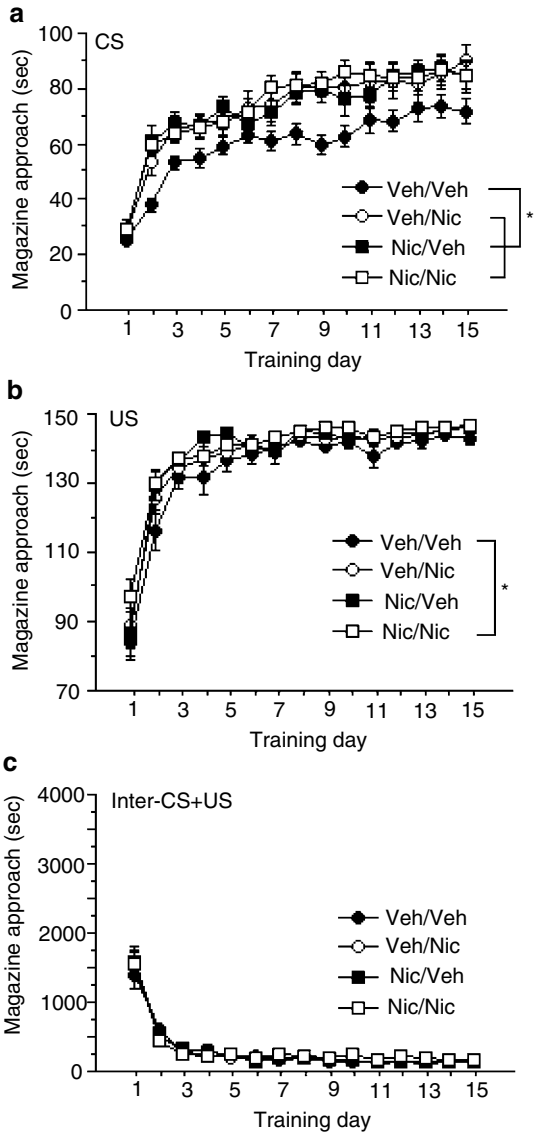

Figure 2 The effect of repeated nicotine (0.35 mg/kg, s.c.) treatment on magazine head entries during CS, US, and inter-CS + US periods in the Pavlovian discriminative approach behavior task. All animals received daily injections with Nic or Veh 15 days prior to the onset of training on Pavlovian discriminative approach behavior as well as after the completion of each of 15 daily training sessions, resulting in the following experimental groups (prior repeated treatment/postsession treatment: Veh/Veh, Veh/Nic, Nic/Veh, and Nic/Nic; $n = 14$ all groups). Repeated nicotine administration according to either of these treatment paradigms increased CS magazine approach, and treatment with nicotine prior to the onset of behavioral testing increased the magazine entries during presentation of the US. Data are presented as the mean total magazine head entries in seconds (± SEM) for daily test sessions. *Indicates a statistically significant group difference (ANOVA followed by Tukey–Kramer's *post hoc* test).

DISCUSSION

The present study investigated the effects of repeated nicotine treatment on Pavlovian discriminative approach

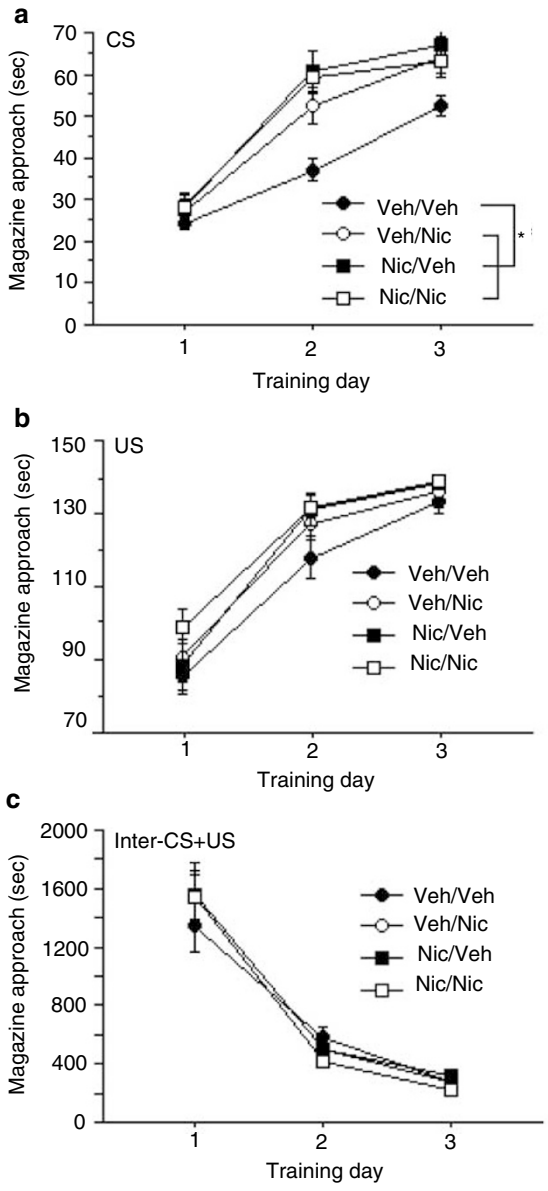

Figure 3 The effect of repeated nicotine (0.35 mg/kg, s.c.) treatment on magazine head entries during CS, US, and inter-CS + US periods in the Pavlovian discriminative approach behavior task during the first 3 days of training. All animals received daily injections with Nic or Veh 15 days prior to the onset of training on Pavlovian discriminative approach behavior as well as after the completion of each daily training session, resulting in the following experimental groups (prior repeated treatment/postsession treatment: Veh/Veh, Veh/Nic, Nic/Veh, and Nic/Nic; $n = 14$ all groups). Repeated nicotine administration according to either of these treatment paradigms increased CS magazine approach, and treatment with nicotine prior to the onset of behavioral testing increased the magazine entries during presentation of the US. Data are presented as the mean total magazine head entries in seconds (± SEM) for daily test sessions. *Indicates a statistically significant group difference (ANOVA followed by Tukey–Kramer's *post hoc* test).

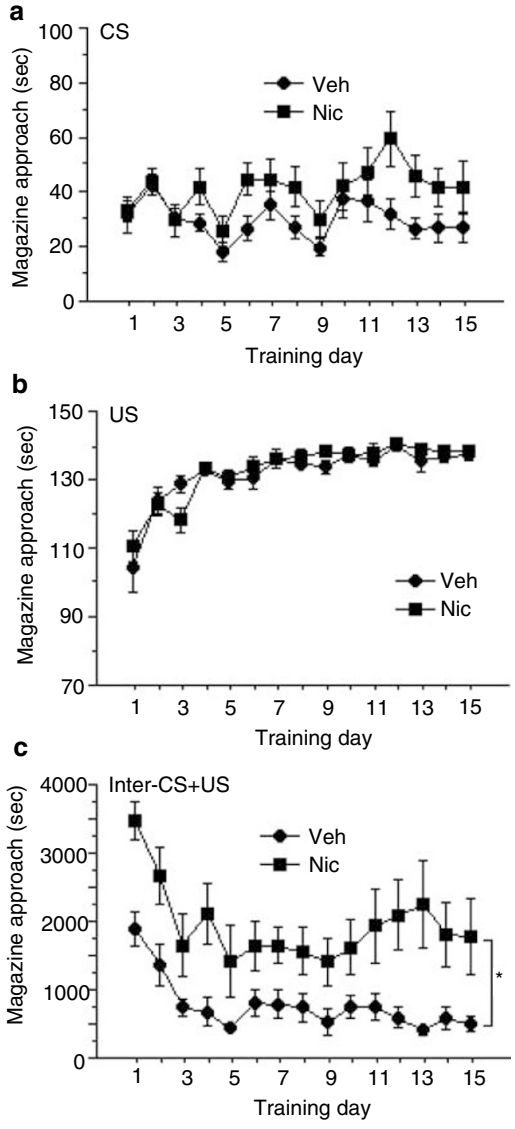

Figure 4 The effect of repeated nicotine (0.35 mg/kg, s.c.) treatment on head entries during CS, US, and inter-CS + US periods in a control task in which the CS and the US are never contingently presented. All animals received daily nicotine ($n = 10$) or vehicle ($n = 10$) injections for 15 days prior to the onset of training. Repeated nicotine administration had no effect of magazine entries during CS or US presentation, but increased magazine head entries during inter-CS + US periods. Data are presented as the mean total magazine head entries in seconds (± SEM) for daily test sessions. *Indicates a statistically significant group difference (ANOVA followed by Tukey–Kramer's *post hoc* test).

behavior in rats. These experiments demonstrate that repeated treatment with nicotine for 15 days prior to reward-related learning, nicotine injections given after each of 15 daily training sessions, or a combination of both prior and postsession treatment, increased head entries into the magazine during presentation of a CS that predicted US availability. These findings confirm previous observations made after prior repeated administration of other psychostimulants (Harmer and Phillips, 1998; Taylor and Jentsch, 2001), and support the hypothesis that repeated nicotine exposures produce long-lasting and functionally significant alterations in neurobiological processes subserving incentive motivation and reward-related learning that are relevant to addiction. These studies also extend previous findings by suggesting that repeated prior drug exposure and repeated postsession injections may produce similar behavioral effects on incentive-motivational processes and reward-related learning.

A control study confirmed that the nicotine-induced enhancement of reward-related learning was specifically because of the predictive relation between the CS and the US. In this experiment, the CS and US were never explicitly paired, and magazine entries during presentation of the CS or the US were unaffected by the prior repeated nicotine treatment. Interestingly, the level of US magazine entries observed in this experiment was not significantly different from the US entries observed when the CS and the US were paired. All animals thus seem to be able to discriminate periods of US availability using subtle cues or occasion setters (eg the sound of the dipper moving). Notably, prior repeated nicotine treatment produced increased responding at other times than when the CS or the US was presented. Since premature magazine entries were punished by a delay of the presentations of both the CS and the US, the increased inappropriate approach behavior could reflect a nicotine-induced impairment in inhibitory control in line with our previous observations (Olausson *et al*, 1999, 2001a–c). It is interesting that the increased 'inappropriate' approach was only observed when the CS and the US were specifically unpaired. However, given the enhanced reward-related learning observed here following repeated nicotine exposure, one possibility is that the inhibitory control deficit is overshadowed by increased learning when the CS and the US is paired, but that the deficit in behavioral control is expressed when the animal is unable to guide behavior through learning of a new Pavlovian association between the CS and the US.

Specific Requirements for Repeated Nicotine Treatment: Potential Mechanisms?

Prior repeated nicotine injections alter the effects of nicotine on a number of neuronal processes and behavioral measures. Most studies have reported that repeated nicotine exposure produces sensitization to these behavioral, neurochemical and molecular effects, including augmented nicotine-induced locomotor activity (Clarke and Kumar, 1983; Clarke *et al*, 1988), elevated nicotine-induced dopamine efflux in the nucleus accumbens (N Acc; Balfour *et al*, 2000), and increased expression of molecular targets including some immediate-early genes (Nisell *et al*, 1997; Pich *et al*, 1997; Mathieu-Kia *et al*, 1998; Ferrari *et al*, 2001). Repeated or chronic nicotine treatment has also been found to increase self-administration of other drugs, including alcohol (Blomqvist *et al*, 1996) and cocaine (Horger *et al*, 1992). Moreover, nicotine typically facilitates learning and cognitive processes (Rezvani and Levin, 2001). To investigate the role of sensitization in the effects of nicotine on reward–reward learning in the Pavlovian discriminative approach behavior paradigm, we treated one experimental group with daily nicotine injections both prior to the onset of training (15 days) as well as giving postsession treatment during the 15 day behavioral testing phase. Combined treatment did not further augment the nicotine-induced enhancement of CS magazine approach compared to rats receiving repeated nicotine treatment prior to the onset of learning or postsession nicotine injections alone, indicating that prior repeated treatment and postsession nicotine injections did not produce synergistic effects in this behavioral paradigm. However, it is also possible that ceiling effects prevented any additional enhancement of Pavlovian discriminative approach behavior in the group receiving both prior and postsession nicotine treatments.

The acquisition of Pavlovian discriminative approach behavior is dependent on processes mediated by cortico-limbic-striatal brain circuits. For example, neurotoxic lesions of the N Acc core and the central nucleus of the amygdala disrupt acquisition of Pavlovian approach behavior (for a review, see Everitt *et al*, 1999; Cardinal *et al*, 2002). These brain regions receive afferent input from the mesocorticolimbic dopamine neurons, and repeated nicotine treatment produce long-lasting neuroadaptations in this dopamine pathway, as well as in other forebrain and cortical areas (Nisell *et al*, 1996; Balfour *et al*, 2000; Di Chiara, 2000; Olausson *et al*, 2001a, b; Pandey *et al*, 2001). Moreover, dopamine neurotransmission in both the N Acc and the amygdala has been implicated in Pavlovian approach behavior (Hitchcott and Phillips, 1998; Parkinson *et al*, 1999; Everitt *et al*, 1999; Phillips *et al*, 2002; Cardinal *et al*, 2002), as well as in the effects of repeated psychostimulant treatment on Pavlovian discriminative approach behavior (Jentsch and Taylor, 1999; Harmer and Phillips, 1999). It is also likely that repeated nicotine injections induce alterations in dopamine-regulated intracellular signaling via the D1/cyclic-Adenosine-Mono-Phosphate (cAMP)/Protein Kinase A (PKA) signaling pathway within these brain circuits, effects that may contribute to the increased approach of the magazine during CS presentation observed in nicotine-treated animals.

The cAMP/PKA signaling pathway, and its downstream molecular targets, influence gene transcription critical for emotional learning (Silva *et al*, 1998; Schafe *et al*, 2001). Indeed, using the present Pavlovian discriminative approach behavior task we have found that direct activation of cAMP/PKA signaling in the amygdala mimics the behavioral effect found in these experiments, as well as the consequences of repeated cocaine administration on Pavlovian approach behavior (Taylor and Jentsch, 2001; Jentsch *et al*, 2002). Given that withdrawal from chronic nicotine treatment upregulates cAMP signaling in the amygdala (Tzavara *et al*, 2002), it seems possible that the enhancement of Pavlovian approach behavior after prior repeated nicotine may occur because of alterations in the dopamine-regulated cAMP/PKA signaling pathway. Since postsession nicotine treatment produced the same results on Pavlovian approach behavior, this raises the possibility that the consequences of postsession or prior repeated nicotine administration produce comparable effects on dopamine-regulated signaling.

Relevance to Stimulus-Control over Behavior

Recent findings have indicated that nicotine augments the influence of CS on instrumental behavior in the nicotine self-administration paradigm. Caggiula and co-workers (2001) demonstrated that a nicotine-associated CS can support instrumental behavior even in the absence of contingent nicotine availability. This result is consistent with our present observations indicating a powerful control over behavior by reward-associated CS in nicotine-exposed animals.

The effect of acute and repeated nicotine treatment on processes related to learning and cognitive functions has been extensively examined (Rezvani and Levin, 2001). However, to the best of our knowledge, few, if any, preclinical studies to date have demonstrated long-lasting facilitation of learning processes after withdrawal from repeated or chronic nicotine administration. We have previously found that repeated exposure to psychostimulants and nicotine may produce concurrent changes in the neural processes involved in incentive-motivation/stimulus control over behavior as well as those involved in cognitive functions, especially inhibitory control (Jentsch and Taylor, 1999; Olausson *et al*, 1999, 2001a–c; Taylor and Jentsch, 2001; Jentsch *et al*, 2002). In combination, these drug-induced effects may result in enhanced control over behavior by reward-associated stimuli and reduced inhibitory modulation of such motivational impulses (Jentsch and Taylor, 1999; Taylor and Jentsch, 2001; Olausson *et al*, 2002), and such nicotine-induced alterations may subserve the behavioral changes observed here. Together these drug-induced alterations are hypothesized to contribute to the compulsive pattern of drug use observed in addiction, but also to cue-induced craving and relapse during short or long periods of drug abstinence. The findings that prior repeated nicotine exposure augments reward-related learning presented here further support the hypothesis that repeated exposure to drugs of abuse, including nicotine, may result in an inability to inhibit reward-elicited behavior indicative of a deficit in inhibitory control functions. Taken together, the present experiments demonstrate that systemic nicotine administration facilitates appetitively motivated stimulus–reward learning in the rat, and suggest that nicotine augments the control over behavior by reward-associated stimuli. These results may be relevant to nicotine abuse since smoking-related cues possess the ability to elicit craving and support smoking behavior in human smokers, and such cues may be important in relapse after nicotine abstinence (Niaura *et al*, 1989; Mucha *et al*, 1998, 1999; Dols *et al*, 2000).

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