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Multiple Risk Factors Precipitate Smoking Relapse: a Demonstration Using a Rat Model of Nicotine Addiction

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ABSTRACT

Relapse to drug addiction behavior in abstinent subjects could be attributable to re-exposure to relevant environments (i.e., environmental cues), drug priming, and stressful life events. In particular, it is common for the abstinent subjects to be imposed with more than one risk factors at any social circumstance. Using a rat model of nicotine addiction, we demonstrated the behavioral effect of exposure to a combination of risk factors on relapse to nicotine-seeking behavior. Male Sprague-Dawley rats were trained to press a level to intravenously self-administer nicotine at a unit dose of 0.03 mg/kg/infusion. In the daily 60-min session, an auditory/visual stimulus was paired with each nicotine infusion so that the stimulus become a nicotine-conditioned cue. After the administration responses were extinguished without nicotine infusion, the reinstatement tests were conducted under five conditions: cue representation, nicotine priming, stress, cue/priming, and cue/stress. The results showed that nicotine priming, pharmacological stressor and re-presentation of the cue respectively reinstated lever responses. The combined presentation of these factors seemed to produce stronger effect. These laboratory findings confirmed the motivational effect of re-exposure to nicotine-associated environmental cues, nicotine priming and confrontation with stressful life events in abstinent smokers. This work would support for behavioral management, in addition to medications, to eliminate exposure to these risk factors.

Introduction

Tobacco smoking and nicotine dependence, like addiction to other drugs of abuse, is a chronic relapsing disorder. Without aids, only 4-7% of adult smokers who try to quit may successfully remain abstinence [1]. Although there have been several pharmacological treatments available, i.e. nicotine replacement therapy, bupropion, and recently varenicline, long-term abstinence rates still remain quite low. The 1-year abstinence rates are 14.6-16.1% for bupropion, 12.9-20.3% for nicotine replacement and 21.9-26.1% for varenicline [2-6]. The high rates of resumption to cigarette smoking in abstinent smokers always present a central and formidable challenge for long-term success of smoking cessation treatment. To develop more effective strategies for prevention of relapse, it is important to fully understand the environmental and neurobiological basis of resumption of smoking behavior in abstinent smokers.

Exposure to drug-associated environmental stimuli (cues), stressful life events, and drug-priming have been thought of as the three major risk factors critically contributing to drug relapse in humans [7-10]. Among these factors, exposure to environmental stimuli previously associated with drug intake might be more liable for the craving for cigarette smoking given that smoking may involve more frequent pairings between the environmental cues and nicotine intake (cigarette puffs, approximately 70,000 times each year) than any other drug-taking behavior, i.e., cigarette smoking may be particularly effective in establishing the incentive properties of nicotine-associated environmental stimuli (cues), such as the smell and taste of cigarettes or contexts within which smoking occurs [11-14].

Facing stressful life events and nicotine priming (a lapse to smoking) usually result in relapse of cigarette smoking and thereby decrease the abstinence rates among smokers [15-19]. In animal studies, although ample evidence has been accumulated showing that these risk factors individually reinstate drug-seeking responding across a wide range of drugs of abuse [20, 21], there has been relatively little effort to characterize resumption of nicotine-seeking behavior in animal models of relapse. Nevertheless, the limited reports on reinstatement of nicotine-seeking in animals have consistently demonstrated the motivational effect of nicotine-associated cues. We [24-26] have showed that reintroduction of nicotine cues after extinction resulted in increased responding on the lever previously reinforced by nicotine delivery. Footshock stress has been documented to reinstate nicotine-seeking in rats [27, 28]. The relapse-eliciting effect of nicotine priming has been reported in some studies [29, 30] but this effect is no consistent [25].

The resumption of addictive behavior including nicotine-seeking during abstinence in humans is often associated with the presence of multiple risk factors [19,31,32]. However, the significance of interactions among these factors for drug-seeking behavior has received little experimental attention. To the best of our knowledge, there have been only a few attempts in animal research. Our previous work has showed that the combination of alcohol-

related cue with footshock stress produced an additive effect on the reinstatement of alcohol-seeking responding in rats [33]. In another study, stress produced by yohimbine administration was found to potentiate the cue-induced reinstatement of cocaine-seeking responses [34]. In terms of nicotine-seeking, there has been no attempt to examine whether stress and cue interact to produce stronger effect. An enhanced effect by the combination of nicotine priming and nicotine cue exposure was observed in one study [35] but not in the other [25].

After systemically characterized the conditioned incentive properties of nicotine-associated cues [22, 23,36,37], in this study we further compared the motivational effects of nicotine cue, nicotine priming, and pharmacological stress as well as explored the effects of combination of cue exposure with nicotine priming or stress in rats. The stressor employed was an anxiogenic drug yohimbine. It is a α2 adrenergic receptor antagonist. It increases activity of noradrenergic systems including neural structures implicated in stress response [38-41] and produces anxiety- and stress-like states in humans and laboratory animals [42-47]. It has been increasingly used as a pharmacological stressor, especially in the field of drug addiction research [34,48-56]. Importantly, yohimbine-induced reinstatement of drug-seeking behavior in a response-reinstatement model of relapse appears to be more robust than that elicited by footshock stress [57-60].

Materials and Methods

Subjects

Twenty two male Sprague-Dawley rats (Harlan) weighing 225–250 g upon arrival were used. Animals were individually housed in a humidity- and temperature-controlled (21-22 oC) vivarium on a reversed light/dark cycle (lights on 7:00 PM; off 7:00 AM) with unlimited access to water. After one week habituation to the colony room, rats were placed on a food-restriction (20 g chow/day) regimen throughout the experiments. Training and experimental sessions were conducted during the dark phase at the same time each day (9:00 AM-3:00 PM). All experimental procedures were carried out in accordance with the *National Institutes of Health Guide for the Care and Use of Laboratory Animals*.

Self-Administration Apparatus

Operant training and reinstatement tests were conducted in operant chambers located inside sound-attenuating, ventilated cubicles (Med Associates, St. Albans, VT). The chambers were equipped with two retractable levers on one side panel and with a 28-V white light above each lever as well as a red chamber light on the top of the chambers. Between the two levers was a food pellet trough. Intravenous nicotine injections were delivered by a drug delivery system with a syringe pump (Med Associates, model PHM100-10 rpm). Experimental events and data collection were automatically controlled by an interfaced computer.

Food Training

One day after the start of the food-restriction regimen, food training sessions began. During the 1-h sessions, lever pressing responses were rewarded with delivery of food pellets (45 mg). Rats could earn a maximum delivery of 45 food pellets first on a fixed-ratio (FR) 1 and then on a FR5 schedule. Successful food training was achieved within 3-5 sessions.

Surgery

After food training, the rats were anesthetized with isoflurane (1-3%) and implanted with jugular catheters. Animals were allowed at least 7 days to recover from surgery. During the recovery period catheters were flushed once/day with 0.1 ml of sterile saline containing heparin (20 U/ml), timentin (10 mg/ml) to maintain catheter patency and prevent infection. Thereafter, the catheters were flushed with the heparinized (20 U/ml) saline prior to and after the experimental sessions throughout the studies.

Nicotine Self-Administration/Conditioning

After recovery from surgery, rats were trained to intravenously self-administer nicotine (0.03 mg/kg/infusion, free base) and associate an auditory/visual cue with nicotine delivery. In the training sessions, animals were placed in the operant conditioning chambers and connected to a drug delivery system. The daily 1-h sessions were initiated by introduction of the two levers with illumination of the red chamber light. Once the FR requirement on the active lever was met, an infusion of nicotine was dispensed in a volume of 0.1 ml in approximately 1 s. Each nicotine infusion was paired with a presentation of the cue consisting of a 5 s tone and illumination of the lever light for 20 s. The latter signaled a 20 s timeout period during which time responses were recorded but not reinforced. Responses on the inactive lever had no consequence. An FR1 schedule was used for days 1-5, an FR2 for days 6-8 and an FR5 for remainder of the experiments. All rats received 30 daily self-administration/conditioning sessions.

Extinction

After completion of the self-administration/conditioning phase, rats were subjected to daily extinction sessions. During the extinction sessions, lever responding was extinguished by withholding nicotine and the cue. Specifically, the daily 1-h extinction sessions began with introduction of the levers and illumination of the red chamber light. Responses on the active lever resulted in the delivery of saline rather than nicotine and the cue presentation was omitted. The criterion for extinction was 3 consecutive days in which the number of responses/sessions was less than 20% of the number of responses/sessions that occurred during the last 3 days of nicotine self-administration.

Reinstatement Tests

One day after the extinction criterion was met, the reinstatement tests were conducted. During the test sessions, responses on the active lever resulted in saline infusion (no availability of nicotine) on the FR5 schedule with a 20 s timeout period. The representation or absence of the cue depending on testing conditions was described below. The tests were scheduled with an interval of 2 days, during which time the daily extinction sessions were performed in order to assure the extinction baseline.

1. Effects of nicotine priming, cue, and their combination

Eleven rats were used for these tests. To test the effect of nicotine priming, rats received a subcutaneous administration of nicotine (0.25 mg/kg in a volume of 1 ml/kg) 5 min before the test session. During the session, there was no cue presentation. To test the effect of cue exposure, the session was conducted with response-contingent re-presentation of the cue. To test the effect of combination of nicotine priming with cue exposure, the session was performed 5 min after nicotine (0.25 mg/kg in a volume of 1 ml/kg) priming injection and with response-contingent re-presentation of the cue during the session.

2. Effect of stress, cue, and their combination

Eleven rats were used for these tests. To test the effect of stress, rats received an intraperitoneal administration of yohimbine (2 mg/kg in a volume of 1 ml/kg) 30 min before the test session. During the session, there was no cue presentation. Then, the effect of cue exposure was tested as described above. To test the effect of combination of stress with cue exposure, the test session was

performed 30 min after yohimbine (2 mg/kg in a volume of 1 ml/kg) injection and with response-contingent re-presentation of the cue during the session.

Data Analyses

Data were presented as the mean (±SEM) number of lever responses. One-way ANOVA was used to separately analyze the data obtained from the two sets of rats (one set for the priming/cue/their combination tests and the other for stress/cue/their combination tests). Differences among individual means in each set of rats were verified by subsequent Newman-Keuls post hoc tests.

Results

Nicotine self-administration/conditioning and extinction

By the end of the 30 daily 1-h self-administration training, rats developed stable operant responding for intravenous nicotine infusions (Figure 1). Averaged across the last 3 sessions, response rates were 83.0±2.9 on the active lever and 9.7±0.9 on the inactive lever, resulting in 14.9±0.5 nicotine infusions per 1-h session. In the first extinction session, rats emitted 68.1±6.4 responses on the active lever and 6.5 ±1.1 on the inactive lever. During the following extinction sessions, lever responses gradually decreased. All rats reached the extinction criterion within 10 daily sessions. Since rats were divided into two sets for the reinstatement tests in a counterbalanced manner, there was no difference in the number of lever responses and nicotine infusions (data not shown).

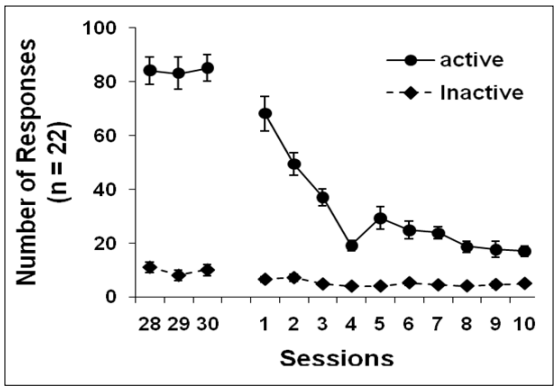

Figure 1: Lever responses made in the last three sessions (sessions 28, 29, 30) of nicotine self-administration/conditioning and during subsequent extinction sessions

Effects of nicotine priming, cue re-presentation, and their combination in the reinstatement tests

A one-way ANOVA on the number of responses emitted on the active lever in the reinstatement test sessions yielded a significant effect of testing condition [$F(3,40)=7.10$, $p<0.001$]. Further Newman-Keuls post hoc tests revealed a significant increase in the number of responses under both the cue exposure ($p<0.01$) and the cue+priming combination ($p<0.05$) conditions above extinction baseline as well as under the cue ($p<0.05$) over the priming condition (Fig. 2). The increase of the active lever responses under the priming condition above the extinction baseline marginally reached statistical significance ($p=0.0602$). However, responses on the inactive lever remained low and indistinguishable between testing conditions (data not shown).

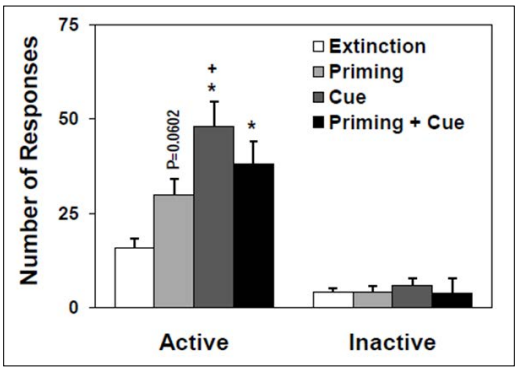

Figure 2: Lever responses in the reinstatement test sessions under the nicotine priming, cue exposure, and their combination conditions. The number of responses is presented as the mean±SEM (n=11). * $P < 0.05$ different from Extinction; + $p < 0.05$ different from Priming

Effects of stress, cue re-presentation, and their combination in the reinstatement tests

A one-way ANOVA on the number of active lever responses collected in the reinstatement test sessions produced a significant effect of testing condition [$F(3,40)=3.97$, $p<0.05$]. Subsequent Newman-Keuls *post hoc* tests showed a significant ($p<0.05$) increase in the number of responses under all three conditions above extinction baseline (Fig. 3). However, responses on the inactive lever remained low and indistinguishable between testing conditions (data not shown).

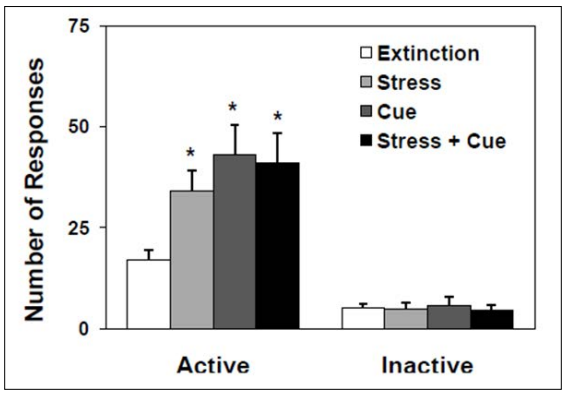

Figure 3: Lever responses in the reinstatement test sessions under the stress, cue exposure, and their combination conditions. The number of responses is presented as the mean±SEM (n=11). * $p < 0.05$ different from Extinction.

Discussion

This study simultaneously examined the motivational effects of the three major risk factors (cue, priming, and stress) under one experimental setting of the response-reinstatement model of relapse and further investigated the issue of whether these factors could interact in reinstating nicotine-seeking behavior. The results demonstrated that re-presentation of the nicotine-associated cue, pharmacological stress produced by yohimbine administration, and nicotine priming injection effectively reinstated extinguished nicotine-seeking behavior in rats. In comparison, re-presentation of the nicotine cue showed the most robust motivational effect. Combination of cue with either priming or stress did not show additive effect.

The issue of whether the cue-induced reinstatement of lever-pressing responses represents conditioned nicotine-seeking behavior needs to be discussed. In the present response-reinstatement paradigm, like in most cases using similar procedures [21], both nicotine and its associated stimulus (cue) were omitted during extinction after self-administration/conditioning training and re-presented in the reinstatement test to determine the conditioned incentive properties of the cue without further nicotine availability. In light of the fact that recent animal studies have shown that some particular sensory stimuli have intrinsic reinforcing values and thereby support moderate levels of operant responding such as lever-pressing [61,24,62,63], it is reasonable to speculate that the increase of responding during re-presentation of the cue might be attributable to the possible reinforcing value of the cue regardless of its association with nicotine; this might then be misinterpreted as response-reinstatement induced by the conditioned incentive properties of the stimulus. To address this concern, in a recent study [22] a control group was included that received procedures exactly the same as other experimental groups with the exception that saline rather than nicotine was available during self-administration/conditioning training. Since the stimulus, albeit the same as in other groups, was never associated with nicotine delivery and subjective actions of nicotine, it did not acquire conditioned reinforcement value. Responding on the active lever in this control group remained low and constant across the self-administration/conditioning, extinction, and reinstatement phases. This finding indicates that the specific stimulus used in that procedure had very little, if any, intrinsic reinforcing value.

Particularly convincing was the observation that removal of the stimulus during extinction in this saline control group did not impact the level of responding. Therefore, the recovery of extinguished responding of nicotine groups during the reinstatement tests resulted from the conditioned incentive value of the stimulus (cue) due to its prior repeated association with nicotine infusion and pharmacological actions. Indeed, by using a procedure that employed criteria established by [64] for demonstrating conditioned reinforcement it was verified that self-administered nicotine endowed its associated stimulus with conditioned reinforcing value [65]. Therefore, it should be no doubt that in the present study nicotine-associated cue-elicited reinstatement of nicotine-seeking responding was solely due to the acquired conditioned incentive properties of the cue because the stimulus cue was exactly the same as in that study [22].

As observed in our previous studies [22,23,36,37], response-contingent re-presentation of the nicotine cue after extinction significantly reinstated responding on the active lever previously reinforced by nicotine. This effect cannot be attributed to the nonspecific arousal effects of reintroduction of the cue because responding on the inactive lever remained at extinction levels. Thus, the reinstatement of responding on the active lever was selectively controlled by the cue that had been associated with delivery and reinforcing actions of nicotine during self-administration/conditioning training. These data lend support for a pioneer endeavor showing that exposing rats to the self-administration training chambers after 21 drug-free days during which time the rats remained in home cages resulted in recovery of lever pressing [66]. The results are in line with increasing evidence in animal studies showing the behavioral significance of nicotine-related cues [66, 24-26]. Taken together, it is indicated that the environmental stimuli associated with availability and subjective actions of nicotine may play an important role in relapse to tobacco smoking in abstinent smokers. Thus, these animal studies lend support for clinical observations that smoking-related cues enhance desire to smoke [67-71].

It has been hypothesized that when compared to other drugs of abuse, environmental stimuli associated with nicotine intake play a more important role in maintaining nicotine self-administration and tobacco smoking [11]. Consistent with this view, tobacco smoking or nicotine self-administration is particularly effective in establishing the incentive properties of accompanying environmental stimuli [11-14] because smoking may involve more frequent pairings between the environmental cues and nicotine intake (cigarette puffs, approximately 70,000 times each year) than any other drug-taking behavior. Moreover, since the reinstatement tests dissociate the motivation to engage in nicotine-seeking behavior from the direct reinforcing properties of nicotine, the procedures used in the present study may be useful for understanding the factors involved in nicotine relapse and the neurobiological mechanisms involved in nicotine-seeking behavior and relapse to tobacco smoking.

Increasing human studies have indicated that a lapse or slip to drug use is one of the best predictors of a full blown relapse to drug abuse including cigarette smoking [73-78]. In several animal studies nicotine priming has been documented to reinstate nicotine-seeking responses [29,30,79]. The present data add to the literature supporting the notion that priming with nicotine leads to recovery of nicotine-seeking. Interestingly, nicotine doses used for the priming tests varied across a wide range from 0.001 mg/kg (intravenous) up to 0.3 mg/kg (subcutaneous). This laboratory dosing matches with clinical definition of lapse that could be any amount of smoking including even only a single puff [73,80]. Together, it is argued that in these priming tests nicotine acts as an occasion setter or discriminative stimulus as long as the injected doses are above a threshold that produces pharmacological actions. This stand could get support from our recent observation showing that self-administered nicotine across a dose range of 0.015-0.06 mg/kg/infusion could endow its associated stimulus similar strength of the conditioned incentive value in rats [22]. However, one study failed to find the response-reinstating effect of priming by intravenous nicotine at 0.01-0.06mg/kg [25]. Although detailed procedural differences in the latter study from other experiments may help explain the inability of nicotine priming to reinstate nicotine-seeking responding, systemic investigation into this issue deserves future efforts.

In the animal model of relapse that tests the effects of stress, footshock has been widely used as a means to induce drug-seeking [81,59,60,10] . Recently, however, a pharmacological stress produced by administration of yohimbine has increasingly been employed instead [34, 49, 50-56] because yohimbine via blockade of the presynaptic α2 adrenergic receptors increases activity of noradrenergic systems including neural structures implicated in stress response and thereby produces anxiety- and stress-like states in humans and laboratory animals [42, 44-47] . In the present study, the yohimbine-produced stress states effectively reinstated extinguished nicotine-seeking responding. This finding is consistent with previous reports that employed footshock as stress [27, 28]. Moreover, the fact that yohimbine has a long half-life (7-8 h) ensured prolonged stress states throughout the whole reinstatement session. That could guarantee the overlap between stress and nicotine cue exposure during the reinstatement sessions.

In contrast to our previous observation showing that stress and ethanol cue interacted to produce additive effect in reinstating

ethanol-seeking [33] and a similar report showing additive response-reinstating effect between yohimbine stress and cocaine cue in reinstating cocaine-seeking [34], combined exposure to stress and nicotine cue in the present study failed to produce an enhanced motivational effect. The magnitude of reinstatement of nicotine-seeking responding under the stress and cue combination was similar to that elicited by nicotine cue alone. The major reason for this discrepancy may reside in the different categories of the drugs of abuse tested across these experiments. In this respect, it is necessary for future work to elucidate the biological mechanisms underlying this discrepancy between nicotine and ethanol or cocaine. Nevertheless, together with the inability of nicotine priming and cue combination to produce a stronger effect above that elicited by nicotine cue alone, these results arrive at an indication that re-exposure to smoking-related environmental cues may play a critical role in the high recidivism rates of smoking. It lends support for the continued effort on cue management as a strategy for the treatment and prevention of smoking relapse.

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