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Nicotine reinstatement of nicotine self-administration after long-term extinction

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Abstract The effect of non-contingent priming injections of nicotine on the reinstatement of drug-seeking behaviour was studied in rats following the long-term extinction of nicotine self-administration. Male rats were trained to lever press for 0.03 mg/kg per infusion of intravenous nicotine. Nicotine maintained a robust self-administration behaviour (11.5 ± 1.2; mean ± SEM infusions/1-h session). When nicotine availability was discontinued, and only a non-contingent saline infusion was presented to the experimental subjects at the beginning of each daily session, responding for the drug-paired lever decreased to low values. After 4–13 sessions, responding extinguished. During this "extinction" period, non-contingent priming infusions of nicotine 0.001, 0.003, 0.01 or 0.03 mg/kg per infusion induced reinstatement of responding for the drug-paired lever. The increased responding, compared with the corresponding previous day on saline, was observed at all four nicotine doses but was not statistically significant for the higher priming dose (0.03 mg/kg per infusion). These preliminary results indicate that nicotine priming is able to induce reinstatement of drug-seeking behaviour in rats similarly to other reinforcing drugs. The present findings show analogies with similar phenomena described in ex-smokers and support the addictive role of nicotine in tobacco smoking.

Key words Nicotine · Priming · Reinstatement · Relapse · Self-administration · Rat

Introduction

Exposure of ex-addicts to the formerly abused drug can reinstate compulsive drug-seeking and drug-taking behaviour even after long periods of abstinence from the drug. The phenomenon (drug "priming" effect) has been described for different classes of drugs of abuse including alcohol, tobacco, psychostimulants and opioids (Ludwig and Wikler 1974; Hodgson et al. 1979; Shiffman 1986; Meyer 1988; Jaffe et al. 1989; Chornock et al. 1992). Several preclinical studies have shown that drugs self-administered by laboratory animals have also the ability to induce the "priming" effect: re-exposure to non-contingent drug infusion reinstated drug-seeking and drug self-administration, i.e. responding for previously drug-paired lever in operant conditioning experiments (Stretch and Gerber 1973; Gerber and Stretch 1975; Davis and Smith 1976; de Wit and Stewart 1981, 1983). "Priming" with drugs that possess similar stimulus properties of the training drug has been shown to reinstate self-administration (Stewart 1984; Stewart et al. 1984; Wise et al. 1990; Stewart and Wise 1992).

The present study was designed to investigate if nicotine priming could reinstate IV self-administration in rats following extinction. Nicotine has been recently regarded as the addictive constituent of tobacco smoking behaviour (US Department of Health and Human Services 1988) because of its reinforcing properties which have been demonstrated in several neurochemical and behavioural studies (Stolerman and Jarvis 1995). In particular, self-administration studies have demonstrated that nicotine maintains robust IV self-administration in rodents (Lang et al. 1977; Corrigall and Coen 1989; Tessari et al. 1995) and in subhuman primates (Goldberg et al. 1983). We used an experimental protocol based on the extinction paradigm which can be considered a reliable and predictive model of human craving and drug-seeking relapse (Markou et al. 1993). Furthermore, we aimed to investigate if possible individual differences in reinstatement of drug-seeking behaviour might be related to the individual behavioural performance and to the past training histories.

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Materials and methods

Subjects

Ten male Wistar rats (Charles River, Calco, Italy) were individually housed in a temperature-controlled environment on a 12-h light: 12-h dark cycle with light on at 6:00 a.m.. At 85% of their free-feeding weights, at which they were maintained, they weighed between 240 and 260 g. Rats were trained or tested once daily. The experiments were always conducted between 7:30 and 12:30 each day from Monday to Saturday. All experiments were performed under the guidelines outlined by the European Council Directive of 24 November 1986 (86/609/EEC).

Apparatus

Behavioural testing was conducted in ten operant chambers (Coulbourn Instruments, Lehigh Valley, USA) encased in sound-insulated cubicles, equipped with ventilation fans (Ugo Basile, Comerio, Italy). Each chamber was equipped with two levers, symmetrically centered on the front panel, located 12.5 cm apart, 2 cm above the grid floor. The food magazine was situated in an opening in the panel between the two levers, 1 cm above the floor. A 2-W white house light was located 26 cm above the food magazine and activated during all the session duration. Right lever presses ("active lever pressing"), corresponding to Fixed Ratio (FR) values required by the schedules of reinforcement, produced the delivery of 45-mg food pellets (Bioserv, USA) or the activation of the infusion pump except during the extinction and priming sessions. Reinforcement delivery was signalled by the 1-s illumination of a 4-W white stimulus light located in the same hole of the food magazine and by the 1-s sounding of a Sonalert device (2.9 Hz, 60 dB). Drug or vehicle solutions were administered via the infusion pump at a volume of 0.022 ml during a 1-s period. Left lever presses did not have any consequence. Lever presses, food pellet and infusion deliveries were recorded. Data acquisition and schedule parameters were controlled by a Med-PC software (Med Associates, Georgia, USA) running on two IBM microcomputer interfaced with the chambers via interface modules (Med Associates).

Training to lever-press

Following a 24-h food deprivation period, rats were trained to lever press for food as a reinforcer. They were gradually trained up to a final Fixed-Ratio = 2 (FR 2) schedule, with a Timeout 60-s (TO 60s) period, 2-h session. Once trained to press the active lever for food reinforcement, rats underwent surgery.

Surgery

The surgical procedure used has already been described by Lane et al. (1992) with minor modifications. Briefly, rats were anaesthetized with a mixture of chlordiazepoxide 9 mg/kg and ketamine 50 mg/kg, 1 ml/kg IP (5 min pretreatment with atropine 1 mg/kg IP), then were implanted with a Silastic catheter (inner diameter 0.012 in, outer diameter 0.025 in, Dow Corning, Michigan, USA) in the right jugular vein. The free end of the catheter was connected to a connector consisting of a modified C313G cannula assembly (Plastic One, Virginia, USA) and the resulting unit was mounted to the skull with dental acrylic cement and fixed via three stainless steel screws. Animals were IV injected with 0.1 ml of a solution containing 1 IU/ml heparin (Liquemin, Roche), 8000 IU/ml streptokinase (Kabikinase, KabiPharmacia, UK) and 65 mg/ml ticarcillin plus clavulanate (Timentin, Zambeletti, Italy). This treatment was repeated every 12 h for 7 days after surgery (period of recovery). Each day after recovery, animals received 0.1 ml of a heparin and 8000 IU/ml streptokinase solution, respectively, before (10 IU/ml heparin) and after (30 IU/ml heparin) the experimental session. On Sundays, catheter patency was kept by flushing only one injection of 0.1 ml solution of 30 IU/ml heparin and 8000 IU/ml streptokinase.

Training to nicotine self-administration

After the period of recovery, rats were trained to nicotine (0.03 mg/kg per infusion) self-administration (FR 1, TO 60 s; session duration up to 25 infusions were delivered, no priming injection). If the animals met the criterion of 25 infusions within the end of the daily session, the FR value was increased to FR 2 and then to FR 3 with session duration up to 15 infusions. This criterion allowed the delivery of a fixed and limited amount of nicotine/session per day. During this phase of the training of nicotine self-administration, the rate of responding could be only measured as average interreinforcer time, calculated by the following formula: total session duration (min)/maximal obtainable infusions (i.e. 15). When rate of responding was stable for at least three consecutive sessions (number of average inter-reinforcer time did not vary more than 20% between three consecutive sessions), session duration was fixed to 1 h, with free access to drug infusion. From this point forward of the training, rate of responding was measured as number of active lever presses/1-h session or as number of nicotine infusions/1-h session.

Extinction and priming tests

Stability on the FR 3 schedule 1-h session (number of active lever presses did not vary more than 20% between three consecutive sessions) was observed after four to eight sessions, eight to 19 total sessions on nicotine self-administration, depending on individual variability. Extinction of the lever pressing for nicotine infusions started when responding for nicotine was stable. The extinction procedure consisted of a 1-h daily session where a non-contingent 1-s infusion of saline was given to animals at the beginning of the session (saline priming). There was no consequence upon responding for the remaining session duration. When the number of active lever presses decreased to a stable low value (extinction period ranging from four to 13 sessions, depending on individual variability), rats were tested for reinstatement of responding after nicotine priming.

Nicotine priming sessions (1 h) were similar to saline priming sessions except for the presence of nicotine solution in the syringe pump instead of saline. Each rat was tested for reinstatement of responding induced by four nicotine doses in nonsystematic order: 0.001, 0.003, 0.01 or 0.03 mg/kg per infusion. At least two stable saline priming sessions elapsed between the nicotine priming sessions. Each animal was tested on as many nicotine priming doses as catheter life allowed: six rats were tested on all the four priming doses, with different orders of nicotine priming.

Drugs

All the drugs were dissolved in heparanized saline (0.09% NaCl + 0.5 UI/ml heparin) and pH adjusted to 7.4 with NaOH. Nicotine unit doses are expressed as mg of free base/kg of body weight per infusion.

Data analyses

Reinstatement of responding was expressed as "increase of responding", i.e. the difference between the active lever presses emitted on

the nicotine priming session and the active lever presses on the previous day on saline condition. Student's t was calculated for comparing the mean values of active lever presses during the saline and the nicotine priming sessions. In order to evaluate the correlation of reinstatement of responding with individual histories of nicotine self-administration performance and self-administration or extinction phase duration, each priming increase of responding (nicotine 0.001, 0.003, 0.01 and 0.03) was analysed with a) the average number of nicotine infusions of the last three nicotine self-administration sessions, b) the average number of active lever presses of the last three nicotine self-administration sessions, c) the number of nicotine self-administration sessions and, d), the number of extinction sessions experienced by the individual animals. Nicotine priming dose/reinstatement relationship was evaluated by means of linear regression analysis (priming dose × increase of responding) by using a SAS-GLM standard procedure. Means, standard errors of the mean (SEM), Student's t-tests and correlation matrix were calculated by using GB-STAT software package (Dynamic Microsystems).

Results

The average number of nicotine infusions under an FR3 schedule of reinforcement was 11.5 ± 1.2 infusions/1-h session (mean ± SEM of the three last nicotine sessions before extinction). Average rate of responding, expressed as number of active lever presses, was 51.4 ± 6.4 lever presses/1-h session (mean ± SEM of the three last nicotine sessions). Responding for inactive lever was low (4.6 ± 1.2 mean ± SEM of the last three nicotine sessions).

After discontinuation of nicotine availability, during the saline priming sessions, responding decreased to 7.2 ± 1.6 active lever presses/1-h session (2.2 ± 1.0 inactive lever presses/1 h; mean ± SEM of the three last sessions).

Reinstatement of responding was observed after the priming infusion of nicotine 0.001, 0.003, 0.01 or 0.03 mg/kg per infusion (Fig. 1). The increase of responding at the four different nicotine doses was not affected by the order of priming, i.e. whether the priming dose was given as first, second, third or last priming. Responding during the saline sessions inserted between the nicotine priming tests rapidly decreased to low values of active lever presses giving a stable "extinction" baseline of responding. Number of inactive lever presses did not significantly differ between nicotine priming compared to saline priming sessions (Fig. 1).

The statistical analysis indicated that increase of responding after nicotine priming was significant at the dose of 0.001, 0.003 and 0.01 mg/kg per infusion (respectively, $P \leq 0.05$, $n = 9$, $P \leq 0.01$, $n = 10$ and $P \leq 0.05$, $n = 9$; Student's t-test for paired data) but not with nicotine 0.03 mg/kg per infusion ($P = 0.21$, $n = 7$; Student's t-test for paired data). At the latter priming dose, 0.03 mg/kg per infusion, four out of seven rats did not increase responding compared to the previous day on saline. Priming with nicotine 0.001 mg/kg per infusion gave the highest average value of reinstatement among the different priming doses. This increase of responding significantly correlated with the average number of nicotine infusions ($r = 0.77$, $P \leq 0.05$) and with the average number of active lever presses ($r = 0.72$, $P \leq 0.05$) emitted by the rats during the three last nicotine self-administration sessions. However, no correlation was found with the number of nicotine self-administration and extinction sessions the animals experienced prior the priming experiments. The increase of responding after the other three nicotine priming doses (0.003, 0.01 or 0.03 mg/kg per infusion) did not correlate with the individual nicotine

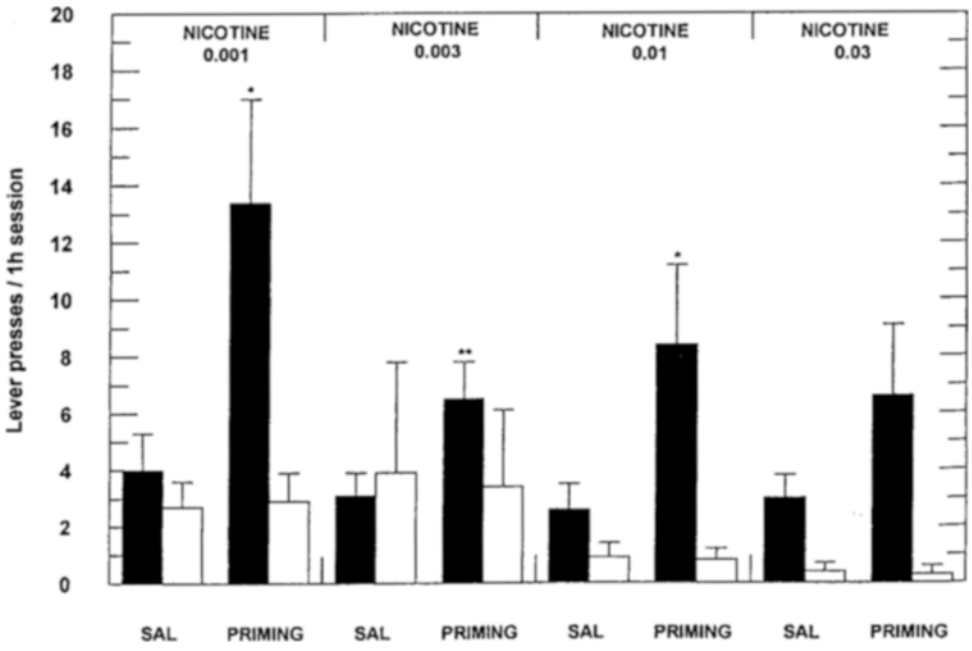

Fig. 1 Effect of non-contingent nicotine priming on reinstatement of responding in rats with an extinguished nicotine self-administration history. *Bars* represent the mean (± SEM) number of active lever presses (*solid bars*) and inactive lever presses (*open bars*) on the nicotine (mg/kg IV; *PRIMING*) or on the previous day saline (*SAL*) priming 1-h sessions. Statistical significance (*$P < 0.05$, **$P < 0.01$) is referred to the comparison (Student's t-test for paired data) between the mean active lever presses on the nicotine priming sessions and on the previous day saline priming sessions. See text for number of rats per group

self-administration performance (average number of nicotine infusions, average number of active lever presses), the number of nicotine self-administrations and the number of extinction sessions.

The effect of changing nicotine priming dose on reinstatement of responding was evaluated on the data obtained from the six subjects receiving all the four nicotine priming doses. Linear regression analysis of priming dose × increase of responding did not indicate any significant dose-response correlation [$F(1, 23) = 0.30$; $P = 0.59$].

Discussion

The present results show that non-contingent IV priming with nicotine reinstated nicotine-seeking behaviour. The results can be summarized as follows: i) nicotine priming-induced reinstatement was observed after a long period (i.e. days) of extinction; ii) reinstatement of responding was specific for the active lever (the one previously associated with nicotine infusion) and significantly higher compared with responding measured during the previous saline priming session; iii) the higher reinstatement was observed with the lowest nicotine priming dose and this effect was individually correlated with previous nicotine self-administration performance; iv) the nicotine self-administration training unit dose, 0.03 mg/kg per infusion, when given non-contingently (priming), did not induce significant reinstatement of responding.

Previously, Corrigall and Coen (1989) showed that nicotine maintained robust self-administration in rats and their results have recently been replicated by our group (Tessari et al. 1995). The finding that nicotine priming is able to reinstate responding in rats with a past history of nicotine self-administration confirms that nicotine possess stimulus control over operant responding for nicotine self-administration. The reexposure of the drug to the animal appeared to induce a motivational state that reinitiates responding for nicotine. The use of the extinction procedure (Stretch et al. 1971; Davis and Smith 1976; Karoly et al. 1978; Shaham et al. 1994) allowed the development of experimental protocols where the expression of the positive incentive value of nicotine could be studied as a specific form of drug-seeking behaviour. With this procedure, our experiments showed that is possible to observe priming-induced reinstatement of self-administration behaviour after several days of extinction. Reinstatement was specific for the drug-paired lever and was not due to general increases in motor activity. Parameters of nicotine self-administration performance, such as the average number of nicotine infusions and active lever presses obtained over three representative sessions, correlated well with the magnitude of reinstatement obtained by priming with the lowest nicotine dose, 0.001 mg/kg per infusion. A possible explanation could be that individual rats with high rates of responding for nicotine self-administration were also more sensitive to the nicotine stimulus and gave higher increases of reinstatement of responding after priming. This correlation, however, was not found with the other three nicotine priming doses. Since the increase of responding did not correlate with the duration of rats' exposure to nicotine self-administration or with the duration of the extinction phase, it could be speculated that these variables reflecting individual experimental history did not influence the nicotine priming effect. However, it must be remembered that most of the reinstatement studies described in literature used different protocols, with within-session extinction (i.e. drug-priming was given only a few hours after discontinuation of the self-administration). Therefore, a comparison between the extent of nicotine priming-induced reinstatement of responding with the one induced by other drugs may be done only with studies using longer periods of extinction (i.e. days). For instance, Shaham et al. (1994) reported that after a saline extinction period of 3–4 days, heroin priming, at a dose usually self-administered by rats, induced an average reinstatement of heroin-seeking behaviour about 3-fold the one induced by saline priming. In the present study, after 4–13 daily extinction sessions, we observed a similar extent of reinstatement with nicotine 0.001 mg/kg priming, the lowest priming dose, but not with nicotine 0.03 mg/kg, the baseline dose for self-administration. Remarkably, nicotine 0.001 mg/kg induced the highest increase in responding.

Our work raised some important arguments that must be discussed. First of all, it is noteworthy that the priming doses used in our study were lower than those used in reinstatement studies for other drugs, which were usually half, equal or higher than the self-administered dose. Secondly, the highest priming dose, 0.03 mg/kg, which is normally used as reinforcing dose during self-administration training, surprisingly did not induce reinstatement. These findings suggest that with the increase of the nicotine priming dose, other effects may appear together with the ability to induce reinstatement of responding. This could also explain the apparent lack of a dose-response relationship in the priming effect of nicotine. Corrigall and Coen (1989) showed that nicotine pre-treatment at a dose reported to stimulate operant responding for food (Corrigall et al. 1988), decreased nicotine self-administration in rats. These authors gave as a possible interpretation the fact that nicotine blood levels might be sufficient for rewarding effect and, therefore, may suppress further lever pressing. Moreover, increasing nicotine content of cigarettes presented to ex-smokers caused a decrease of urge to smoke (i.e. craving) (Shiffman and Jarvik 1976). It appeared that as the priming dose of the drug become close to a rewarding dose, the intensity of subjective craving diminishes

and this may be in contrast with a "proponent-process" theory of relapse, which states that it is a drug-like process, rather than a drug-opposite one, that stimulate motivated behaviour (Stewart et al. 1984). A possible explanation is that higher priming doses decrease responding early in the 1-h session period and, therefore, there could be a temporally shifted increase of responding, as shown in reinstatement studies with other drugs (Stewart and Wise 1992). In our study, however, analysis of cumulative pattern of responding over a 1-h session after nicotine 0.03 mg/kg priming were not able to show reinstatement, even later in the session. More studies are needed in order to show if a longer session duration may allows to observe a delayed reinstatement of responding induced by nicotine 0.03 mg/kg. On the other hand, the interpretation of data obtained with nicotine priming doses higher than 0.03 mg/kg could be difficult, since nicotine doses higher than the self-administration baseline dose induced behavioural suppression in our experimental conditions (unpublished data) or significant decrease of responding, presumably because of aversive effects, as reported by Corrigall and Coen (1989). In conclusion, differences of extinction duration (as above discussed) or the different way nicotine self-administration is regulated compared to the self-administration of other drugs (Corrigall 1992) may help to explain how the effect of nicotine priming is evident at a different dose range compared to other drug priming.

Our results, even if preliminary, add further evidence to the similarities between nicotine and other reinforcing drugs such as psychomotor stimulants, opiates and alcohol. These drugs, which have been demonstrated to be addictive substances, are abused by human and are self-administered by experimental animals under different protocols and schedules of reinforcement. Moreover, using similar experimental paradigms, non-contingent priming injection of these drugs has been shown to reinstate responding in animals with a past history of drug-taking. This drug stimulus-dependent effect showed several analogies with the phenomenon of drug reexposure-induced relapse in ex-addicts. Our results, taken together with the recent reports on the reinforcing properties of nicotine (for a review, see Stolerman and Jarvis 1995), strongly support the evidence that the drug has similar features to those of other drugs of abuse.

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