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# Nicotine and heroin augment cocaine-induced dopamine overflow in nucleus accumbens

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**Abstract**

The current public debate on nicotine concentrates on the abuse potential of nicotine per se. However, little is known about the interaction of nicotine with other drugs of well-established abuse liability such as cocaine. Indeed, cigarette smoking increases the intake of cocaine and other drugs of abuse. In order to test if these epidemiological data are reflected in a neurochemical correlate of the reinforcing effects of drugs of abuse, i.e., dopamine overflow in the nucleus accumbens, in vivo brain microdialysis was used to examine the effects of nicotine and cocaine either alone or in combination in freely moving rats. Furthermore, the effects of the nicotine + cocaine combination were compared to another drug combination of high abuse potential, i.e., heroin + cocaine ('speedball'). Both nicotine + cocaine as well as heroin + cocaine stimulated nucleus accumbens dopamine overflow in an additive manner. Repeated intermittent administration of nicotine did not significantly alter the effects of a subsequent challenge with the nicotine + cocaine combination. These data suggest that the clinical-epidemiological findings on either drug combination are reflected in a stimulatory interaction on nucleus accumbens dopamine overflow that is additive. No significant tolerance seems to develop to this effect of nicotine. These neurochemical findings support behavioral data suggesting that the reinforcing effects of cocaine and heroin are additive and predict that nicotine will enhance the reinforcing effects of cocaine. © 1997 Elsevier Science B.V.

*Keywords:* Additivity; Cocaine; Dopamine; Heroin; (Interaction); Microdialysis; Nicotine; Nucleus accumbens; Speedball; Synergism

## 1. Introduction

The current public debate on nicotine concentrates on the abuse potential of nicotine per se. However, little is known about potential interactions between nicotine and other drugs of well-established abuse liability, such as cocaine. Epidemiological studies suggest that cigarette smoking increases the intake of cocaine and other drugs of abuse (i.e., marijuana, alcohol, heroin, and lysergic acid diethylamide (LSD); (Schorling et al., 1994; Konings et al., 1995) and that, vice versa, cocaine users consume more cigarettes than nonusers (Budney et al., 1993; Higgins et al., 1994; but see Radzius et al., 1997). Animal self-administration studies have shown that prior exposure to nicotine, which serves as a reinforcer on its own (Lang et al., 1977; Goldberg and Henningfield, 1988; Stolerman and Shoaib, 1991), increases the rate of acquisition of cocaine self-administration (Horger et al., 1992).

At the neuroanatomical level, nicotine is known to activate acetylcholine receptors, many of which are located presynaptically on dopaminergic projections from the ventral tegmental area to the nucleus accumbens (Stolerman and Shoaib, 1991). The resulting release of dopamine into the synaptic cleft can be measured by in vivo microdialysis as an increased dopamine overflow in the nucleus accumbens in freely moving rats (Corrigall et al., 1994; Nisell et al., 1994), a neurochemical effect that is shared by a variety of other drugs of abuse (Di Chiara and Imperato, 1988a; Pontieri et al., 1996). Thus, nucleus accumbens dopamine overflow represents a robust though not specific (Abercrombie et al., 1989; Tidey and Miczek, 1996) neurochemical correlate of the reinforcing effects of nicotine and other drugs of abuse. Cocaine is also known to increase nucleus accumbens dopamine overflow (Di Chiara and Imperato, 1988a) through a mechanism that is quite distinct from that of nicotine, i.e., through inhibition of a dopamine transporter responsible for the reuptake of dopamine from the synaptic cleft (Ritz et al., 1987). Therefore, a synergistic action of nicotine and cocaine on nucleus accumbens dopamine overflow is to be expected

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from their different modes of action. The present study was designed to investigate if nicotine indeed facilitates cocaine's stimulatory action on nucleus accumbens dopamine overflow, both when nicotine is given acutely as well as after repeated intermittent administration, which in terms of human use is the more relevant pattern of intake (Higgins et al., 1994). Testing the effect of the repeated intermittent administration of nicotine on a subsequent challenge with nicotine and cocaine is also of interest as the intermittent mode of administration has been suggested to lead to nicotine sensitization (Horger et al., 1992; ElBizri and Clarke, 1994).

The nicotine + cocaine combination was compared with a drug combination of demonstrated high abuse potential, i.e., heroin + cocaine ('speedball'; Kosten et al., 1987). Interestingly, self-reports of polydrug abusers indicate that the 'rush' induced by heroin (accompanied by sedation and relaxation) is qualitatively different from that induced by cocaine (accompanied by stimulation), although both are perceived as pleasurable (Ellinwood et al., 1976; Seecof and Tennant, 1986; Foltin and Fischman, 1992). Heroin is reportedly combined with cocaine to alleviate the aversive effects of the 'crash' after a cocaine rush, especially when the abuser intends to curb craving for cocaine at the end of a 'binge' (Kosten et al., 1987; Foltin and Fischman, 1992). These anecdotal data have been confirmed in a human behavioral laboratory setting by Foltin and Fischman (1992): Self-reports of 'high' and 'liking' using visual analog scales showed subadditivity for a morphine + cocaine combination; 'high' scores remained elevated for a longer period of time when the combination was administered. The effects of the combination on estimates of the 'street value' of the drug injection (i.e., an indirect measure of its reinforcing effect) showed pure additivity in the intermediate dose range. Thus, the present study also examined the nature and extent of the heroin + cocaine combination on nucleus accumbens dopamine overflow. Opioids have been shown to increase nucleus accumbens dopamine overflow by disinhibition of dopaminergic protons from the ventral tegmental area to the nucleus accumbens (Spanagel et al., 1992; Di Chiara and North, 1992), a mechanism of action that is different from that of either cocaine or nicotine (see above). Therefore, heroin — like nicotine — is expected to facilitate cocaine's stimulatory action on nucleus accumbens dopamine overflow. The present study represents, to our knowledge, the first demonstration of the effects of both the heroin + cocaine and the nicotine + cocaine combination on nucleus accumbens dopamine overflow.

## 2. Materials and methods

### 2.1. Animals

Male Wistar rats weighing 275–350 g at the beginning of the experiment were housed in groups of three under a 12 h light–dark cycle (lights on at 7.00 h) at 23°C. After implantation of the microdialysis probe, the animals were housed individually and sacrificed at the end of the experiment to determine probe location. All procedures have been approved by the University of British Columbia Committee on the Use of Laboratory Animals.

### 2.2. Surgery and in vivo microdialysis procedure

Rats were implanted under pentobarbital (60 mg/kg) and xylazine (2 mg/kg) anesthesia with microdialysis probes aimed at the nucleus accumbens. The coordinates of the probe tip (with a 2 mm active dialysis membrane) relative to bregma were AP +3.5 mm, ML +1.5 mm, and DV −7.8 mm (incisor bar at +5 mm) according to the atlas of Pellegrino et al. (1981). Microdialysis experiments were performed 48 h after implantation of the analytical probes.

Dopamine and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were measured in the nucleus accumbens of freely moving rats by in vivo microdialysis and on-line high-pressure liquid chromatography using electrochemical detection as detailed previously (Wilson et al., 1995). Briefly, concentric microdialysis probes (membrane: Filtral$^{R}$ AN69; 40 000 molecular cutoff; inner diameter, 0.24 mm; Hospal-gambro) were perfused with a 1 mM sodium phosphate-buffered solution containing 147 mM NaCl, 3 mM KCl, 1 mM $MgCl_2$, and 1.3 mM $CaCl_2$ (pH 7.3) at 5 μl/min. The dialysate was collected in 10-min bins and subjected to HPLC (mobile phase: 100 mM sodium acetate adjusted to pH 4.1 with acetic acid, 0.6 mM octanesulfonic acid, 0.01 mM $Na_2EDTA$, and 120 ml methanol/liter; column: Nucleosil 5C18).

### 2.3. Data analysis

Only animals with a confirmed probe tip location within the nucleus accumbens as defined by the atlases of Pellegrino et al. (1981) and Paxinos and Watson (1986) were included in the study. After levels of dopamine and metabolites had stabilized (variation < 15%), the mean of three consecutive 10-min samples was defined as 100% baseline and all subsequent dialysate levels normalized to it. Statistical comparisons (Analysis of Variance with Huynh-Feldt adjustment to account for repeated measures; Systat) included the last sample before a treatment and all samples taken after that treatment as shown in the figures. Unless indicated otherwise, all shown values are means ± SEM of $n$ animals. The numbers of animals contributing to each treatment group were: saline, 6; nicotine, 6; cocaine, 5; heroin, 6; acute nicotine + cocaine, 11; nicotine + cocaine in saline-pretreated animals, 9; nicotine + cocaine in nicotine-pretreated animals, 8; heroin + cocaine, 9.

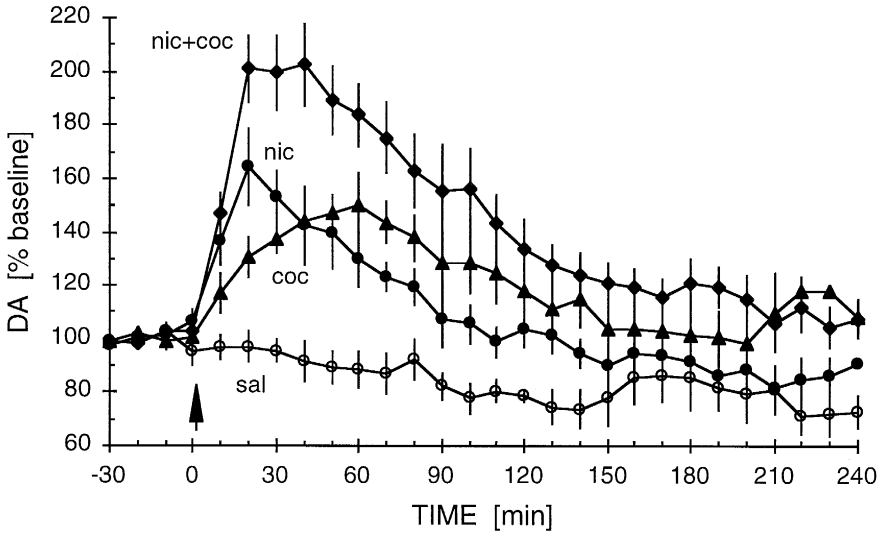


Fig. 1. Effect of nicotine and cocaine either alone or in combination on the amount of dopamine in the dialysate obtained from rat nucleus accumbens. Dialysate was collected in 10 min bins; thus, the symbol at time point zero refers to the sample collected from −10 min to immediately before the injection of saline or drugs. Dialysate was obtained in 6 rats injected s.c. with saline (open circles), 6 rats injected with 0.1 mg/kg (−)-nicotine base (injected as dihydrogen tartrate salt; filled circles), 5 rats injected with 3 mg/kg cocaine HCl (triangles), and 11 rats injected with a combination of nicotine and cocaine (diamonds). Dialysate dopamine levels were determined by high-pressure liquid chromatography with electrochemical detection (HPLC-ED) and are expressed as % of baseline dialysate dopamine levels determined in the first three 10 min bins (fmol/min: saline, 3.4 ± 0.6; nicotine, 2.7 ± 0.4; cocaine, 3.1 ± 0.8; nicotine + cocaine, 3.0 ± 0.6).

### 2.4. Drugs and dosage regimen

Drugs were dissolved in physiological saline. The pH of (−)-nicotine dihydrogen tartrate was adjusted to that of saline (i.e., 5.85) by addition of NaOH. All injections were given subcutaneously into the flank. The saline-treated animals received saline injections into both flanks (sal + sal); the animals treated with a single drug received the drug injection into one flank and a saline injection into the other flank (nic + sal, coc + sal, her + sal); drug combination animals received one drug into each flank (nic + coc, her + coc). Drug doses are expressed in mg/kg for the HCl salts of cocaine and heroin (both from BDH, Toronto, Ontario, Canada) and the (−)-nicotine base (Sigma, St. Louis, MO, USA). The dosage regimen for the intermittent repeated administration of nicotine was identical to that

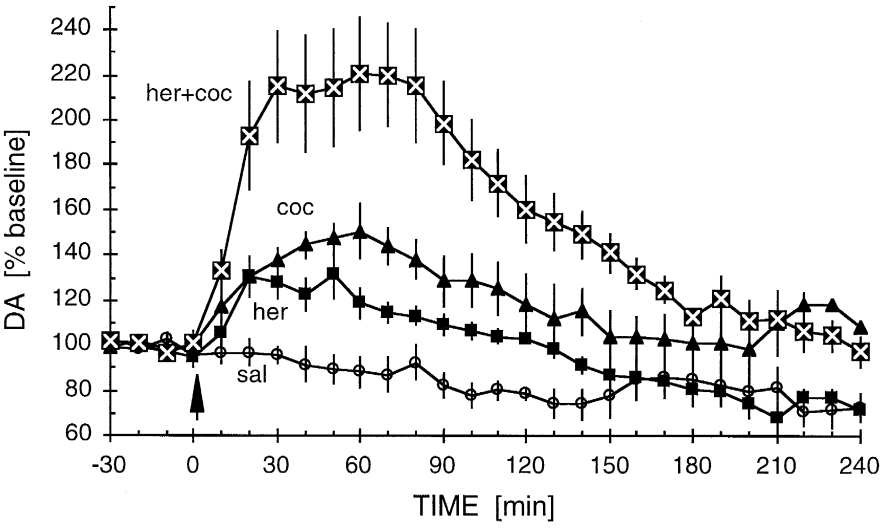


Fig. 2. Effect of heroin and cocaine either alone or in combination on the amount of dopamine in the dialysate obtained from rat nucleus accumbens. For conventions see Fig. 1. Rats were injected s.c. with saline (open circles), 0.15 mg/kg heroin HCl (squares), 3 mg/kg cocaine HCl (triangles), or a combination of heroin + cocaine (crossed-in squares). Dopamine baseline dialysate levels were (in fmol/min): heroin, 3.7 ± 0.6 ($n = 6$), heroin and cocaine, 3.1 ± 0.6 ($n = 9$). For other baseline values see Fig. 1.

employed by ElBizri and Clarke (1994): Rats were injected with either saline (sat pt) or 1 mg/lcg nicotine (nic pt) once between 08.00 and 10.00 h and once between 16.00 and 18.00 h for 11 days. Nicotine was injected for the last time between 08.00 and 10.00 h on day 12; microdialysis experiments were started 24 h later. Thus, either saline or 1 mg/kg (−)-nicotine base was injected twice daily for a total of 11.5 days.

## 3. Results

In microdialysis experiments performed in freely moving rats, subcutaneous injections of 0.1 mg/kg nicotine, 0.15 mg/kg heroin, or 3 mg/kg cocaine significantly increased the amount of dopamine (Figs. 1 and 2) and its metabolites DOPAC and HVA (Figs. 3 and 4) recovered from the NAC (see below), while baseline overflow of dopamine, DOPAC, or HVA was not significantly different between saline and treatment groups (one factor analysis of variance (ANOVA); $P = 0.95$ for dopamine and $P = 0.98$ for DOPAC and HVA). The respective values for the saline group were (in fmol/min; $n = 6$): dopamine, $3.4 \pm 0.6$; DOPAC, $483 \pm 102$; HVA, $229 \pm 46$. In the animals treated with saline alone, a slight rundown of the dopamine signal was observed (Fig. 1). In contrast, nucleus accumbens dopamine overflow increased and peaked 60 min after cocaine administration, and 20 min after administration of nicotine (Fig. 1) or heroin (Fig. 2).

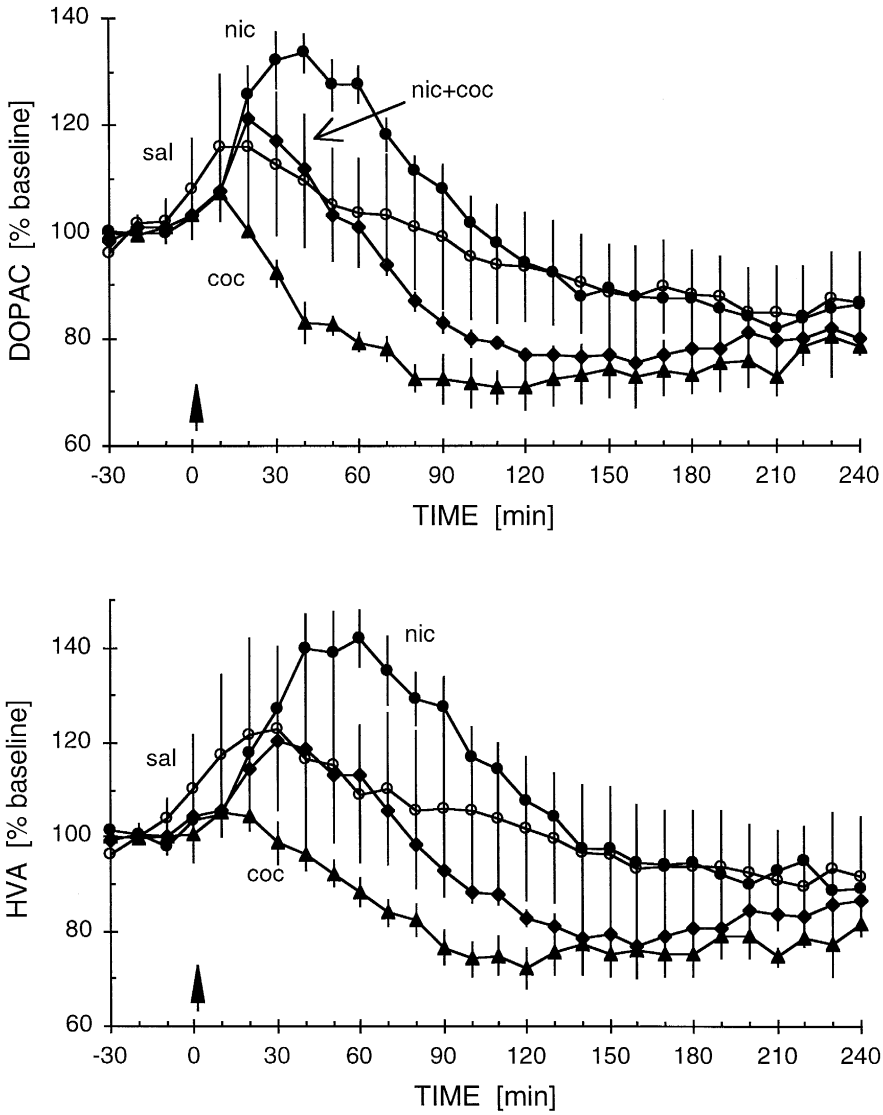


Fig. 3. Effect of nicotine and cocaine either alone or in combination on the amount of DOPAC and HVA in the dialysate obtained from rat nucleus accumbens. For conventions see Fig. 1. Rats were injected s.c. with saline (open circles), 0.1 mg/kg (−)-nicotine base (injected as dihydrogen tartrate salt; filled circles), 3 mg/kg cocaine HCl (triangles), or a combination of nicotine and cocaine (diamonds). Dialysate was assayed for 3,4-dihydroxyphenylacetic acid (DOPAC, upper panel) and homovanillic acid (HVA, lower panel). DOPAC baseline dialysate levels were (in fmol/min): saline, $483 \pm 102$; nicotine, $532 \pm 95$; cocaine, $528 \pm 97$; nicotine + cocaine, $466 \pm 31$. HVA baseline dialysate levels were (in fmol/min): saline, $229 \pm 46$; nicotine, $228 \pm 25$; cocaine, $228 \pm 37$; nicotine + cocaine, $204 \pm 11$.

Simultaneous administration of nicotine and cocaine further increased the overflow of dopamine (Fig. 1). The increase was even more pronounced for the heroin + cocaine condition (Fig. 2). Combining cocaine with either nicotine or heroin shifted the time of peak nucleus accumbens dopamine overflow to earlier time points after the injections (from 60 min to 20–30 min). Two-factor ANOVA of the dopamine data (0–240 min after the drug or saline injection) revealed significant group ($P < 0.001$) and time ($P < 0.001$) effects as well as a significant group × time interaction ($P < 0.001$). The same was true for DOPAC (group, $P = 0.004$; time, $P < 0.001$; group × time $P < 0.001$) and HVA (group, $P = 0.001$; time, $P < 0.001$, group × time, $P < 0.001$). A comparison of the nucleus accumbens dopamine overflow (i.e., group × time) for the individual treatment groups showed significant differences between saline and nicotine ($P < 0.001$), saline and cocaine ($P = 0.001$), and saline and heroin ($P = 0.002$). The nicotine + cocaine combination gave a nucleus accumbens dopamine overflow that was significantly different from nicotine ($P = 0.012$) and cocaine ($P = 0.049$). Similarly, the nucleus accumbens dopamine overflow for heroin + cocaine combination was significantly different from that for heroin ($P = 0.025$). The difference in the nucleus accumbens dopamine overflow between the heroin + cocaine combination and cocaine approached statistical significance ($P = 0.15$).

The additive effects of the nicotine + cocaine and heroin + cocaine combination were also obvious on a qualitative level when analyzing the dopamine metabolite time patterns: Nicotine (Fig. 3) and heroin (Fig. 4) produced an initial increase in the overflow of DOPAC and HVA that

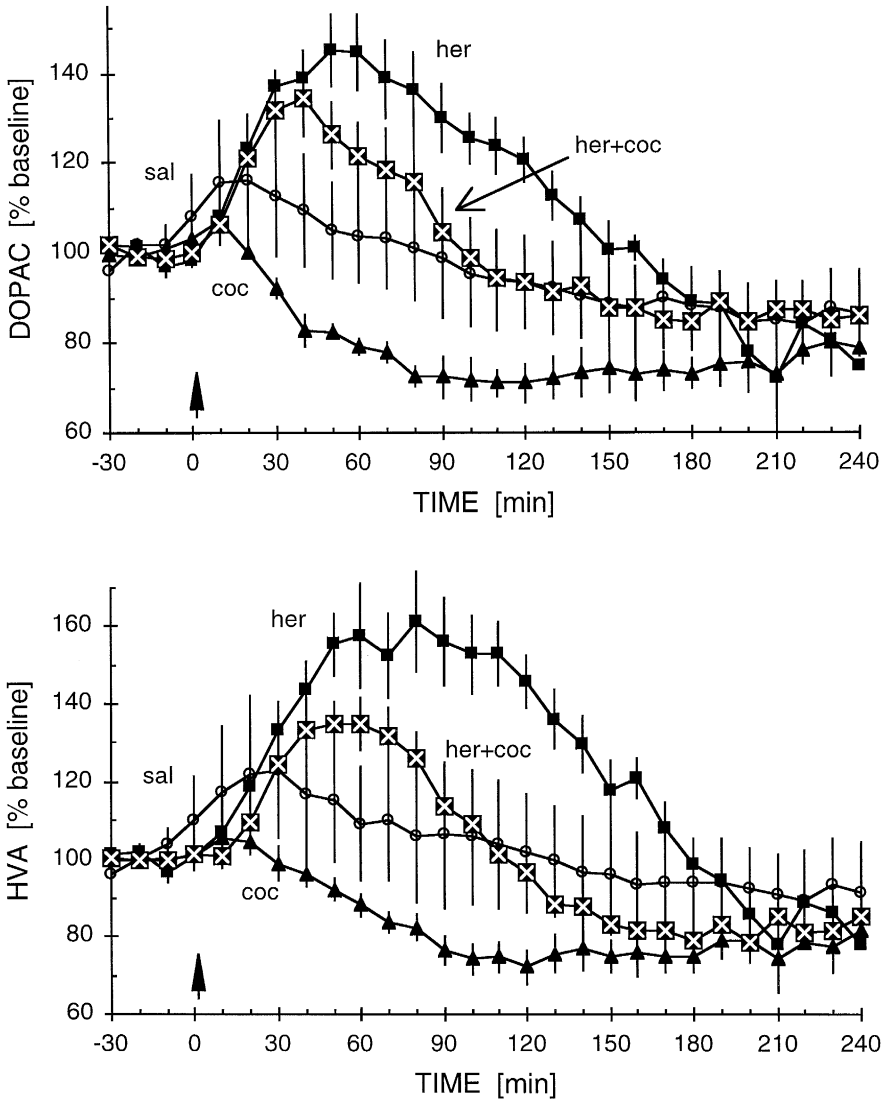


Fig. 4. Effect of heroin and cocaine either alone or in combination on the amount of DOPAC and HVA in the dialysate obtained from rat nucleus accumbens. For conventions see Fig. 1. Rats were injected s.c. with saline (open circles), 0.15 mg/kg heroin HCl (squares), 3 mg/kg cocaine HCl (triangles), or a combination of heroin and cocaine (crossed-in squares). DOPAC baseline dialysate levels were (in fmol/min): heroin, 500 ± 84 ($n = 6$), heroin + cocaine, 487 ± 66 ($n = 9$); HVA baseline levels were: heroin, 212 ± 45, heroin + cocaine, 220 ± 23. For other baseline values see Fig. 1Fig. 2.

is typical for these drugs of abuse and distinguishes them from cocaine (Di Chiara and Imperato, 1988a,b); cocaine led to the expected immediate decrease in DOPAC and HVA. The combination of both nicotine + cocaine (Fig. 3) and heroin + cocaine (Fig. 4) resulted in a biphasic pattern that lay almost exactly between the time pattern of the singly administered drugs.

When expressed as the drug-saline difference between the normalized signals (thus correcting for the dopamine signal rundown seen after saline), the dopamine signal observed after the nicotine + cocaine combination was essentially the same as the calculated combined dopamine signal of the drugs given alone, indicating that the effect of the nicotine + cocaine combination is purely additive (Fig. 5, top panel). In contrast, the observed dopamine signal of the heroin + cocaine combination was slightly larger than the calculated sum of the individual dopamine responses for either drug (Fig. 5, bottom panel). Standard errors, however, overlapped for the majority of the time points tested. To further test for statistical significance, areas under the curve ($AUC_{0-120min}$) were determined for each individual animal in each drug group. These AUCs were

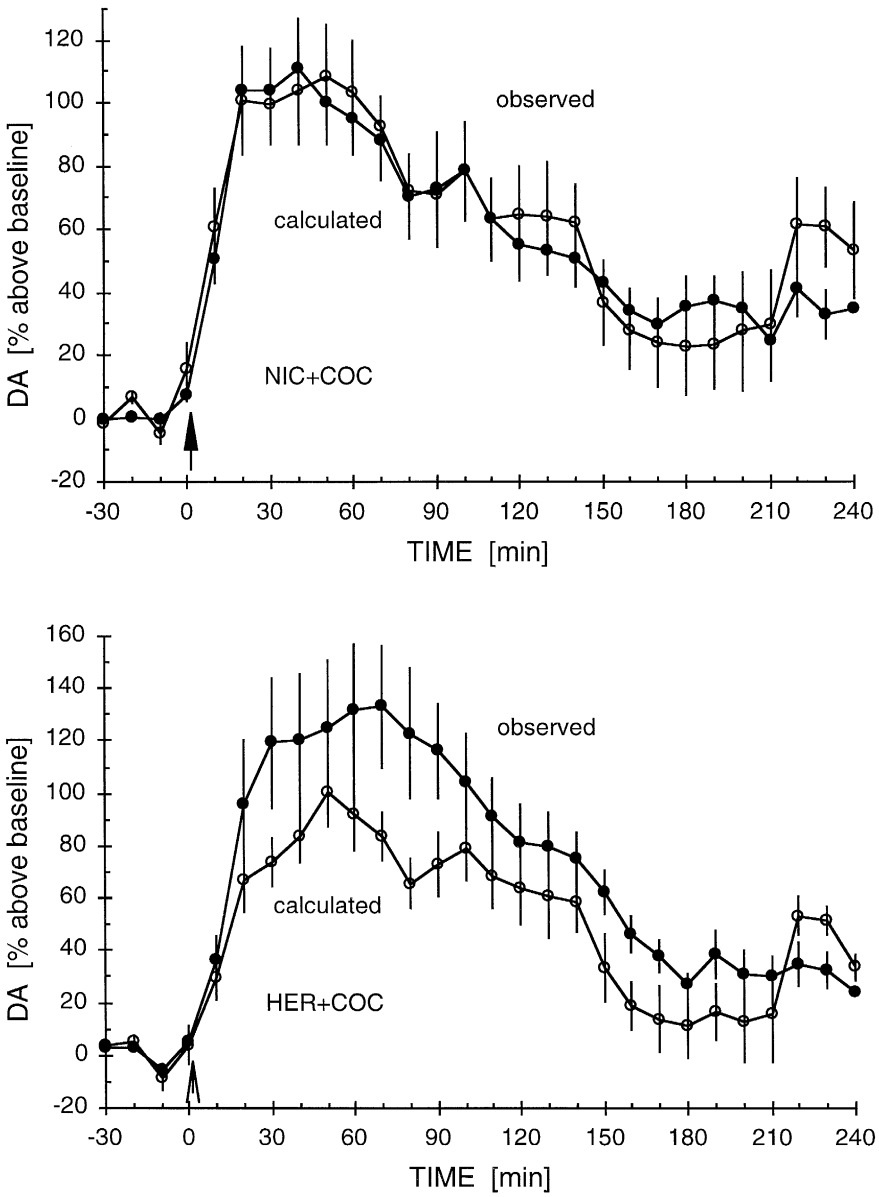


Fig. 5. Comparison of the calculated and experimentally observed effects of the nicotine/cocaine and heroin + cocaine combinations on nucleus accumbens dopamine overflow. For comparison, the % baseline values for each combination (as shown in Figs. 1 and 2) had to be transformed in the following way: First, the average values for the saline group were subtracted from each animal of the drug group (either single drug or drug combination) to obtain the baseline-corrected drug effect for each animal (hence the term '% above baseline'). Then animals of the single drug groups were randomly combined and their effects added; so were the variances of the single drug effects. From the added variances, standard errors were calculated on the bases of one group (i.e., $n = 5$). These calculated combined values (open circles) are directly compared to the experimentally observed values of the true drug combination (filled circles). Shown are means ± SEM. Upper panel, nicotine + cocaine combination; lower panel, heroin + cocaine combination.

then randomly combined among individuals of the single drug groups and compared to the observed $AUC_{0-120min}$ for the drug combination. The values were (means ± SEM; $AUC_{0-120min}$ expressed as cumulative % baseline drug minus cumulative % baseline saline): Nicotine, 545 ± 81; cocaine, 555 ± 82; heroin, 345 ± 57; nicotine + cocaine calculated, 1100 ± 118 vs. observed, 999 ± 151; heroin + cocaine calculated, 900 ± 132 vs. observed, 1284 ± 243. Accordingly, 2-tailed *t*-tests yielded a $P$ value of 0.61 for the difference between the calculated and observed nicotine + cocaine combination and $P = 0.19$ for the heroin + cocaine combination. All these findings show that the effect of nicotine and cocaine on nucleus accumbens dopamine overflow is purely additive, while the combined effect of heroin and cocaine might be supraadditive without, however, reaching statistical significance.

The combined effect of nicotine and cocaine could also be observed when animals were pretreated with repeated intermittent injections of saline or 1 mg/kg nicotine (twice daily for 11.5 days). It should be noted that the s.c. injection of 1 mg/kg nicotine resulted in observable respiratory (deep, low-frequency breathing) and locomotor (hyperactivity) effects in most rats, especially during the first injections. Fig. 6 shows that in the saline-pretreated animals, the amount of the nucleus accumbens dopamine increase by a subsequent simultaneous injection of 0.1 mg/kg nicotine and 3 mg/kg cocaine was slightly larger than when administered to drug-naive animals (Fig. 6; see also Fig. 1). In contrast, when the nicotine + cocaine combination was given to rats that had been repeatedly challenged with nicotine, its effect seemed to be slightly smaller than in drug-naive animals. Thus, it appears that the repeated intermittent nicotine injections resulted in a slight tolerance to nicotine. However, differences in nucleus accumbens dopamine levels between treatment groups were not significant (group, $P = 0.14$; time, $P < 0.001$; group × time, $P = 0.29$). The differences were even less pronounced for the overflow of DOPAC and HVA (data not shown). Interestingly, both repeated saline injections (sat pt) and nicotine injections (nic pt) appeared to decrease the baseline overflow of dopamine. In contrast, the baseline overflows for DOPAC and HVA were increased in sat pt animals and decreased in nic pt animals. However, none of the changes reached statistical significance (one-factor ANOVA; $P = 0.11$ for dopamine, $P = 0.06$ for DOPAC, $P = 0.11$ for HVA).

## 4. Discussion

The effects of nicotine and cocaine as well as the effects of heroin and cocaine on the overflow of dopamine and its metabolites DOPAC and HVA in the nucleus accumbens of freely moving rats were of an additive nature. Thus, the effects of the nicotine + cocaine combination on nucleus accumbens dopamine overflow as a neurochemical correlate of the reinforcing effects of drugs of abuse corresponded well with epidemiological data on nicotine showing an increased rate of cocaine abuse (see Section 1). Similarly, the neurochemical data on the heroin + cocaine combination are in good agreement with quantitative data obtained in the human behavioral laboratory which showed that the reinforcing effects of the cocaine and heroin (as indirectly measured by asking the subjects

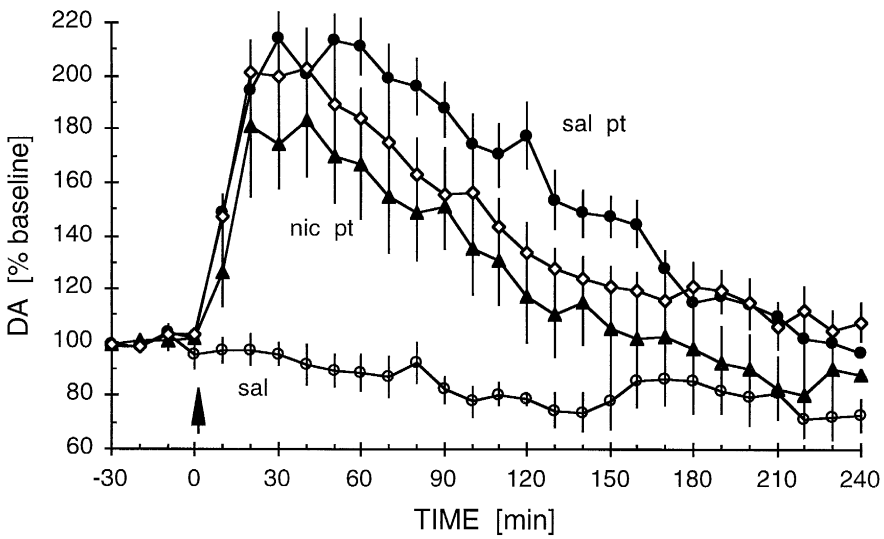


Fig. 6. Effect of repeated intermittent nicotine administration on the nucleus accumbens dopamine overflow induced by a nicotine + cocaine combination. Rats were injected twice daily for 11.5 days with either saline (filled circles) or 1 mg/kg (−)-nicotine base (injected as dihydrogen tartrate salt; triangles) and challenged 24 h after the last injection with a combination of 0.1 mg/kg (−)-nicotine and 3 mg/kg cocaine HCl. The resulting increase in nucleus accumbens dopamine overflow is compared to drug-naive animals acutely challenged with the nicotine + cocaine combination (open diamonds; see Fig. 1) or saline (open circles; see Fig. 1). Baseline DOPAMINE values were (in fmol/min): saline-pretreated animals, 1.6 ± 0.3 ($n = 9$); nicotine-pretreated animals, 1.7 ± 0.6 ($n = 8$). For other baseline values and conventions see Figs. 1 and 2.

to assign a 'street value' to the administered doses) were additive (Foltin and Fischman, 1992). In contrast, the additive effect of the heroin + cocaine combination on nucleus accumbens dopamine overflow seemed to be more pronounced than the subadditive effects of this combination on self-reports of pleasure ('high'; Foltin and Fischman, 1992). The present study, however, does not explain the special attractiveness of the heroin + cocaine combination ('speedball') to drug users that results from combining two qualitatively different drug effects (Kosten et al., 1987; Foltin and Fischman, 1992). In particular, we did not observe any prolongation of the nucleus accumbens dopamine overflow that could be predicted from drug addicts' self-reports that heroin coadministration prolonged the cocaine 'high' (Foltin and Fischman, 1992). There are also no known pharmacokinetic interactions between cocaine and heroin in humans (Goodman Gilman et al., 1990; Winger et al., 1992) that could explain the special attractiveness of 'speedballs' for addicts. The only indication of a qualitative change in time-course of the dopamine response produced by the heroin + cocaine or nicotine + cocaine combination came from the dopamine metabolites: The immediate decrease in DOPAC and HVA which is typical for cocaine was changed into an initial increase, especially by coadministration of heroin.

The findings of the present study predict an additive effect of either nicotine or heroin on the reinforcing properties of cocaine in humans or animals working under operant schedules. Self-administration behavior under most operant schedules is characterized by a biphasic dose–response curve (Johanson and Schuster, 1981; Young and Herling, 1986; Winger et al., 1989; Meisch and Lemaire, 1993). This biphasic dose–response relationship begins with an ascending part at lower unit doses (i.e., increasing the dose available for self-administration increases responding or the willingness to work to obtain the drug), followed by a descending part at higher doses (i.e., increasing the available dose even further decreases responding). Thus, an increase in the reinforcing effect of cocaine by nicotine or heroin would result in a shift of the whole cocaine dose–response curve to the left, i.e., responding for low cocaine doses (and, thus, cocaine intake) would increase whereas responding for high cocaine doses (and, thus, cocaine intake) would decrease relative to conditions under which only cocaine was available (see Fig. 4 of Mello and Negus (1996) for an excellent graphic representation). The maximum amount of cocaine taken, however, would be lower in presence of a drug enhancing cocaine's reinforcing effect. The fact that nicotine appears to increase the intake of cocaine and other drugs of abuse suggests that human cocaine abuse occurs at the ascending part of the cocaine dose–effect curve, i.e., that submaximal doses are self-administered, at least in the college student population that was surveyed (Schorling et al., 1994).

The respective data from animals working under operant schedules for cocaine and/or heroin are inconsistent: In rhesus monkeys, progressive ratio schedules revealed supraadditivity (Rowlett and Woolverton, 1996) whereas no additivity was found using a second-order schedule of reinforcement used in a different laboratory (Mello et al., 1995). In rats working under a fixed-ratio schedule, the addition of heroin produced a dose-dependent and predominantly downward shift of the descending part of the cocaine dose–response relationship which was interpreted as an additive reinforcing effect (Hemby et al., 1996). As to the effects of nicotine on the reinforcing effect of cocaine, the only available study (Horger et al., 1992) showed that prior exposure to 9 daily nicotine injections subsequently increased the rate of acquisition of cocaine self-administration in rats, suggesting that nicotine might increase the propensity of the organism to take cocaine. Further experimental work is required to resolve the apparent discrepancies for the heroin + cocaine combination as well as to provide a larger body of experimental evidence for the effects of nicotine + cocaine combinations on behavior. The present data clearly predict, however, that nicotine will enhance the reinforcing effects of cocaine.

The essentially additive effect of the heroin + cocaine combination on nucleus accumbens dopamine overflow differs from previous results obtained in our laboratory with a buprenorphine + cocaine combination which clearly produced a supraadditive effect (Brown et al., 1991). In that study, however, buprenorphine produced a time-course of nucleus accumbens dopamine overflow that was very different from that generated by heroin (Fig. 2): Buprenorphine increased nucleus accumbens dopamine overflow in a continuous linear fashion up to the last point tested (i.e., 5 h), whereas heroin produced an increase in nucleus accumbens dopamine overflow that peaked at 20 min and returned to pre-heroin levels within 3 h. Nevertheless, the basis of this discrepancy remains to be determined.

ElBizri and Clarke (1994) have shown that intermittent nicotine (1 mg/kg twice daily for 11.5 days) upregulates [$^3$H]nicotine binding sites by 16–36%, depending on the brain region investigated. We utilized this schedule in an attempt to optimize conditions for a demonstration of possible nicotine sensitization on the level of nucleus accumbens dopamine overflow but could not demonstrate any sensitization. These results are consistent with earlier failures to show functional sensitization, both by other groups (Benwell and Balfour, 1992; Horger et al., 1994) as well as our own (Damsma et al., 1989). Thus, the slight upregulation of [$^3$H]nicotine binding sites (ElBizri and Clarke, 1994) was not reflected in functional changes at the level of dopamine neurons (present study). It seems as if the same mechanisms of the nicotine + cocaine interaction apply to the chronic as well as the acute situation.

In conclusion, this study shows that nicotine — regardless of its own status in the abuse liability debate — enhances an important neurochemical effect of cocaine, a drug of undisputed high abuse potential. In addition, the present experiments provide neurochemical documentation

that there are significant interactions between cocaine and heroin. The neurochemical evidence for the additive nicotine + cocaine interaction calls for a detailed investigation of this phenomenon at the behavioral level.

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## References

Abercrombie, E.D., Keefe, K.A., DiFrischia, D.S., Zigmond, M.J., 1989. Differential effect of stress on in vivo dopamine release in striatum, nucleus accumbens, and medial frontal cortex. J. Neurochem. 52, 1655.

Benwell, M.E.M., Balfour, D.J.K., 1992. The effects of acute and repeated nicotine treatment on nucleus accumbens dopamine and locomotor activity. Br. J. Pharmacol. 105, 849.

Brown, E.E., Finlay, J.M., Wong, J.T.F., Damsma, G., Fibiger, H.C., 1991. Behavioral and neurochemical interactions between cocaine and buprenorphine: Implications for the pharmacotherapy of cocaine abuse. J. Pharmacol. Exp. Ther. 256, 119.

Budney, A.J., Higgins, S.T., Hughes, J.R., Bickel, W.K., 1993. Nicotine and caffeine use in cocaine-dependent individuals. J. Subst. Abuse 5, 117.

Corrigall, W.A., Coen, K.M., Adamson, K.L., 1994. Self-administered nicotine activates the mesolimbic dopamine system through the ventral tegmental area. Brain Res. 653, 278.

Damsma, G., Day, J., Fibiger, H.C., 1989. Lack of tolerance to nicotine-induced dopamine release in the nucleus accumbens. Eur. J. Pharmacol. 168, 363.

Di Chiara, G., Imperato, A., 1988a. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc. Natl. Acad. Sci. USA 85, 5274.

Di Chiara, G., Imperato, A., 1988b. Opposite effects of mu and kappa opiate agonists on dopamine release in the nucleus accumbens and in the dorsal caudate of freely moving rats. J. Pharmacol. Exp. Ther. 244, 1067.

Di Chiara, G., North, R.A., 1992. Neurobiology of opiate abuse. Trends Pharmacol. Sci. 13, 185.

ElBizri, H., Clarke, P.B.S., 1994. Regulation of nicotinic receptors in rat brain following quasi-irreversible nicotinic blockade by chlorisondamine and chronic treatment with nicotine. Br. J. Pharmacol. 113, 917.

Ellinwood, E.H., Eibergen, R.D., Kilbey, M.M., 1976. Stimulants: Interaction with clinically relevant drugs. Ann. N.Y. Acad. Sci. 281, 393.

Foltin, R.W., Fischman, M.W., 1992. The cardiovascular and subjective effects of intravenous cocaine and morphine combinations in humans. J. Pharmacol. Exp. Ther. 261, 623.

Goldberg, S.R., Henningfield, J.E., 1988. Reinforcing effects of nicotine in humans and experimental animals responding under intermittent schedules of i.v. drug injection. Pharmacol. Biochem. Behav. 30, 227.

Goodman Gilman, A., Rall, T.W., Nies, A.S., Tayler, P. (Eds.), 1990. Goodman's and Gilman's The Pharmacological Basis of Therapeutics, 8th ed. Pergamon Press, New York, NY.

Hemby, S.E., Smith, J.E., Dworkin, S.I., 1996. The effects of eticlopride and naltrexone on responding maintained by food, cocaine, heroin and cocaine/heroin combinations in rats. J. Pharmacol. Exp. Ther. 277, 1247.

Higgins, S.T., Budney, A.J., Hughes, J.R., Bickel, W.K., Lynn, M., Mortenson, A., 1994. Influence of cocaine use on cigarette smoking. JAMA 272, 1724.

Horger, B.A., Giles, M.K., Schenk, S., 1992. Preexposure to amphetamine and nicotine predisposes rats to self-administer a low dose of cocaine. Psychopharmacology 107, 271.

Horger, B.A., Valadez, A., Wellman, P.J., Schenk, S., 1994. Augmentation of the neurochemical effects of cocaine in the ventral striatum and medial prefrontal cortex following preexposure to amphetamine, but not nicotine: An in vivo microdialysis study. Life Sci. 55, 1245.

Johanson, C.E., Schuster, C.R., 1981. Animal models of drug self-administration. In: Mello, N.K. (Ed.), Advances in Substance Abuse. JAI Press, Greenwich, CT, p. 219.

Konings, E., Dubois-Arber, F., Narring, F., Michaud, P.A., 1995. Identifying adolescent drug users: Results of a national survey on adolescent health in Switzerland. J. Adolesc. Health 16, 240.

Kosten, T.R., Rounsaville, B.J., Kleber, H.D., 1987. A 2.5-year follow-up of cocaine use among treated opioid addicts. Have our treatments helped?. Arch. Gen. Psychiatry 44, 281.

Lang, W.J., Latiff, A.A., McQueen, A., Singer, G., 1977. Self administration of nicotine with and without a food delivery schedule. Pharmacol. Biochem. Behav. 7, 65.

Meisch, R.A., Lemaire, G.A., 1993. Drug self-administration. In: van-Haaren, F. (Ed.), Techniques in The Behavioral and Neural Sciences. Elsevier, Amsterdam, p. 257.

Mello, N.K., Negus, S.S., 1996. Preclinical evaluation of pharmacotherapies for treatment of cocaine and opioid abuse using drug self-administration procedures. Neuropsychopharmacology 14, 375.

Mello, N.K., Negus, S.S., Lukas, S.E., Mendelson, J.H., Sholar, J.W., Drieze, J., 1995. A primate model of polydrug abuse: Cocaine and heroin combinations. J. Pharmacol. Exp. Ther. 274, 1325.

Nisell, M., Nomikos, G.G., Svensson, T.H., 1994. Infusion of nicotine in the ventral segmental area or the nucleus accumbens of the rat differentially affects accumbal dopamine release. Pharmacol. Toxicol. 75, 348.

Paxinos, G., Watson, C., 1986. The Rat Brain in Stereotaxic Coordinates. Academic Press, New York, NY.

Pellegrino, L.J., Pellegrino, A.S., Cushman, A.J., 1981. A Stereotaxic Atlas of The Rat Brain. Plenum Press, New York, NY.

Pontieri, F.E., Tanda, G., Orzi, F., Di Chiara, G., 1996. Effects of nicotine on the nucleus accumbens and similarity to those of addictive drugs. Nature 382, 255.

Radzius, A., Carriero, N.J., Weinhold, L.L., Gorelick, D.A., Henningfield, J.E., 1997. Changes in cigarette smoking not observed following repeated cocaine self administration. Exp. Clin. Psychopharmacol. 5, 51.

Ritz, M.C., Lamb, R.J., Goldberg, S.R., Kuhar, M.J., 1987. Cocaine receptors on dopamine transporters are related to self-administration of cocaine. Science 237, 1219.

Rowlett, J.K., Woolverton, W.L., 1996. Self-administration of cocaine and heroin combinations under a progressive-ratio schedule in rhesus monkeys. NIDA Res. Monogr. 162, 171.

Schorling, J.B., Gutgesell, M., Klas, P., Smith, D., Keller, A., 1994. Tobacco, alcohol and other drug use among college students. J. Subst. Abuse 6, 105.

Seecof, R., Tennant, F.S., 1986. Subjective perceptions to the intravenous 'rush' of heroin and cocaine in opioid addicts. Am. J. Drug Alcohol Abuse 12, 79.

Spanagel, R., Herz, A., Shippenberg, T.S., 1992. Opposing tonically active endogenous opioid systems modulate the mesolimbic dopaminergic pathway. Proc. Natl. Acad. Sci. 89, 2046.

Stolerman, I.P., Shoaib, M., 1991. The neurobiology of tobacco addiction. Trends Pharmacol. Sci. 12, 467.

Tidey, J.W., Miczek, K.A., 1996. Social defeat stress selectively alters mesocorticolimbic dopamine release: An in vivo microdialysis study. Brain Res. 721, 140.

Wilson, C., Nomikos, G.G., Collu, M., Fibiger, H.C., 1995. Dopaminergic correlates of motivated behavior: Importance of drive. J. Neurosci. 15, 5169.

Winger, G., Palmer, R.K., Woods, J.H., 1989. Drug-reinforced responding: Rapid determination of dose–response functions. Drug Alcohol Dep. 24, 135.

Winger, G., Hofmann, F.G., Woods, J.H., 1992. A Handbook on Drug and Alcohol Abuse. Oxford University Press, New York, NY.

Young, A.M., Herling, S., 1986. Drugs as reinforcers: Studies in laboratory animals. In: Goldberg, S.R., Stolerman, I.P. (Eds.), Behavioral Analysis of Drug Dependence. Academic Press, Orlando, FL, p. 9.