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A Rodent Model of Cocaine Abstinence Syndrome

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MALIN, D. H., W. D. MOON, E. T. MOY, R. E. JENNINGS, D. M. MOY, R. L. WARNER AND O. B. WILSON. *A rodent model of cocaine abstinence syndrome.* PHARMACOL BIOCHEM BEHAV **66**(2) 323–328, 2000.—This study introduces a rat model of cocaine abstinence syndrome based on quantitation of spontaneously emitted behaviors following termination of continuous drug exposure (analogous to established methods of assessing morphine and nicotine abstinence). Groups of eight male S-D rats were infused SC for 7 days via an osmotic minipump with saline alone or with 40 or 60 mg/kg/day cocaine HCl. Pumps were removed and rats were observed at 12, 24, 36, and 48 h postremoval. Each 15-min observation employed a checklist of abstinence signs including ptosis, chews, teeth chatters, gasps, writhes, seminal ejaculations, head shakes, and tremors. The high infusion rate group displayed significantly more signs than the low infusion rate group, which in turn, displayed significantly more signs than the saline group. Cocaine injection significantly reduced signs by 83.3%, while saline injection reduced them by only 4.9%. In another experiment, rats infused with 60 mg/kg/day showed significantly more signs 36 h postinfusion than before infusion, during infusion and 84 h postinfusion. Finally, 6.5 days of infusion resulted in significantly more abstinence signs than did 1.5 days of infusion. This rapid and simple model quantitated cocaine abstinence syndrome in a manner that was cocaine-reversible and related to the rate and duration of drug infusion. © 2000 Elsevier Science Inc.

Cocaine dependence Cocaine abstinence syndrome Withdrawal syndrome Rat

COCAINE users may display an abstinence syndrome involving symptoms such as dysphoria, fatigue, sleep and activity disturbances, vivid unpleasant dreams, and increased appetite (1). There have also been reports of depression, nervousness, irritability, anhedonia, and drug craving. These symptoms can develop within hours of withdrawal, and may last for weeks before disappearing (10,40,49). Withdrawal syndromes may contribute to relapse (25), because the greatest risk of return to cocaine use occurs during this period (50). Rat models of opiate abstinence (11) and nicotine abstinence (30) have proven to be useful for exploring neurobiological mechanisms of drug dependence and evaluating experimental therapeutic approaches. Analogous models of cocaine dependence seemed feasible because cessation of chronic cocaine exposure apparently results in an altered state of the rat nervous system. Withdrawal from continuous cocaine infusion in rats has resulted in altered sensitivity to the behavioral stimulant effects of cocaine and various dopamine and serotonin receptor antagonists (21–24). Cocaine withdrawal also induced elevated intracranial self-stimulation thresholds (35), caused an increase in certain startle responses (36) and a proconflict effect in the conditioned suppression of drinking paradigm (8). However, there have been very few studies of spontaneously emitted behavioral abstinence signs following cocaine withdrawal in rats.

The present study introduces a rapid and simple model for inducing and quantitating cocaine abstinence syndrome in the rat. Cocaine dependence was induced by 7 days of continuous subcutaneous infusion of cocaine HCl via osmotic minipump. A standard checklist of behavioral abstinence signs, based on checklists for opiate and nicotine abstinence signs (11,30), was employed to assess cocaine abstinence syndrome. A major methodological problem in the observational approach to assessing drug withdrawal is that different subjects tend to display their irritation or distress through different signs. In the case of morphine and nicotine dependence, cumulating occurrences of signs across all categories has resulted in sensi-

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tive indicators of drug abstinence. These cumulative indices have proven their sensitivity by responding in an orderly way to differences in drug infusion rate, time of observation, renewed exposure to drug, and drug antagonist-precipitated withdrawal (7,16,27,29,30,33). Based on pilot experiments, the cocaine abstinence checklist was modified from morphine and nicotine checklists only through a more restricted definition of "writhes" and "shakes" (described in the Method section).

Four experiments were performed to determine whether this model met the following validity criteria for a measure of drug abstinence syndrome. 1) There should be more abstinence signs following cocaine infusion than following saline vehicle infusion. 2) There should be more signs following high infusion rates than low infusion rates. 3) Abstinence signs should be potently alleviated by cocaine injection. 4) There should be more abstinence signs in the days immediately following cocaine withdrawal than before cocaine infusion, during cocaine infusion, or following a subsequent recovery period. 5) A longer duration of infusion should result in more subsequent abstinence signs. Additionally, locomotor activity levels were observed during periods of cocaine infusion and abstinence.

EXPERIMENT 1: THE EFFECT OF COCAINE INFUSION RATE ON SUBSEQUENT ABSTINENCE SYNDROME

Method

Subjects were 24 male Sprague–Dawley (S-D) rats weighing 380–420 g and maintained on a 12 L:12 D cycle and ad lib food and water. Rats were observed for abstinence-like signs prior to implantation. Rats showing more than seven signs over 15 min were not used in this study. This was done to eliminate the few potential subjects that had a tendency to express abstinence-like behaviors independent of drug exposure. Each rat was implanted SC with one Alzet 2ML1 osmotic minipump under halothane anesthesia. A large, loose subcutaneous pocket for the pump was created with a rounded spatula to reduce the chance of local necrosis from tight contact between the pump outlet and adjacent skin. Three randomly assigned groups of eight rats were then infused SC for 7 days with saline alone, 40 mg/kg/day cocaine HCl (RBI, Natick, MA) in saline, or with 60 mg/kg/day cocaine HCl in saline. These infusion rates were chosen because they were in the general range administered in previous studies concerning the chronic effects of continuous subcutaneous cocaine infusion (23,26,37). There have been conflicting reports as to whether subcutaneous cocaine injection causes dermal necrosis in the rat (5,6). However, cocaine has been continuously subcutaneously infused at 20 mg/kg/day for 2 weeks and 50 mg/kg/day for 7 days without mention of tissue necrosis in one case (37) and, in another case, with no observable evidence of necrotic skin or subcutaneous lesions (26).

After 7 days of infusion, the osmotic minipumps were removed under halothane anesthesia in the first hour of the light phase. Animals and solutions were coded and each rat was observed for 15 min under "blind" conditions in a clear plastic observation chamber. The same core group of observers scored each rat in all experiments reported in this article. Observations were conducted at 12, 24, 36, and 48 h following pump removal. The initial observation was conducted 12 h after pump removal to allow the thorough clearance of cocaine and halothane anesthetic, and to allow the rat time to recover from the surgical procedure to remove the pump. Twelve rats (four per treatment group) were additionally observed at 84 and 156 h after pump removal. Observers counted occurrences of signs utilizing a standard checklist of abstinence signs modified slightly from morphine and nicotine abstinence checklists on the basis of small pilot experiments with cocaine-dependent rats. Signs included ptosis, teeth chatter/chews, gasps/writhes, head shakes/tremors, and miscellaneous, less frequent signs such as seminal ejaculations and hind foot scratches. A writhe is a contraction of the trunk lasting at least a second. "Stomach lift writhes" are defined as writhes limited to constriction of the ventral abdomen. These were not counted in the case of cocaine abstinence because they failed to differentiate cocaine-treated from saline-treated rats in the pilot experiments. "Body shakes" are defined as a series of rapidly repeated rotational movements involving the body trunk. These were not counted in the case of cocaine abstinence, because, unlike head shakes, they did not differentiate the treatment groups. Ptosis is counted no more than once per minute so as to prevent extremely high scores resulting from periods of continuous ptosis. This discrete counting method results in ptosis counts of roughly comparable magnitude to other major signs (Fig. 2). Each rat's overall abstinence score was the number of instances of abstinence signs cumulated across all categories. At the end of the experiment, each rat was sacrificed by overdose of halothane. The pump was then removed, and the interior and exterior of the nearby skin was examined for any necrosis.

Results

Figure 1 shows overall abstinence signs as a function of the three infusion rates and the six observation times. The 12 through 48-h observation data, which included all 24 rats, was analyzed by ANOVA with one repeated-measures variable. ANOVA revealed a significant infusion rate effect, $F(23, 72) = 12.48$, $p < 0.01$, no significant trial effect, $F(23, 72) = 0.38$, NS, and no significant interaction effect (infusion rate $\times$ trial), $F(23, 72) = 0.60$. The number of signs (mean $\pm$ SEM) averaged over the trials from 12 through 48 h following pump removal was 5.97 $\pm$ 1.51 for the saline group, 12.13 $\pm$ 2.63 for the 40 mg/kg day group, and 17.84 $\pm$ 1.73 for the 60 mg/kg/day group. Linear trend analysis revealed a significant posi-

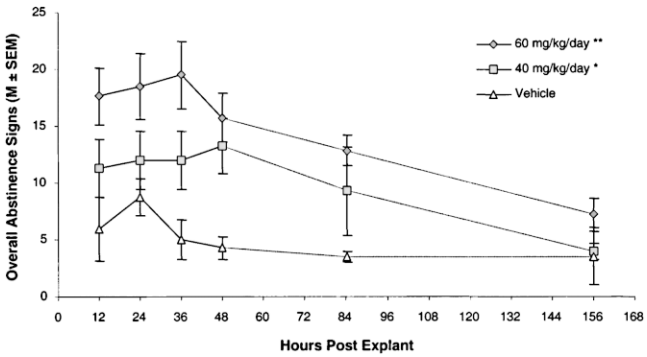

FIG. 1. Overall abstinence signs (mean $\pm$ SEM) as a function of the three infusion rates (60, 40, and 0 mg/kg/day cocaine HCl in saline) and the six observation times following termination of infusion. Note that eight rats per treatment group were observed through 48 h (solid lines), while only four of these per group were also observed at 84 and 156 h (dotted lines). *$p < 0.05$ vs. saline group over 12–48 h, **$p < 0.01$ vs. saline group, and $p < 0.05$ vs. 40 mg/kg/day group over 12–48 h.

tive trend of abstinence signs as a function of infusion rate, $F(1, 19) = 9.55$, $p < 0.01$. Post hoc analysis (LSD test) revealed that the 40 mg/kg/day group had significantly more signs than saline infused rats, $p < 0.05$, while the 60 mg/kg/day group had significantly more signs than the saline group, $p < 0.01$, as well as the 40 mg/kg/day group, $p < 0.05$. Figure 2 shows individual categories of signs displayed by each group, cumulated over the 12 through 48-h period. Dunnett's test also revealed that the 60 mg/kg/day cocaine HCl group differed significantly from saline-infused controls in ptosis, $p < 0.01$, chews/teeth, $p < 0.05$, and misc. signs, $p < 0.05$. The difference in gasps/writhes approached significance, $0.05 < p < 0.10$. The 40 mg/kg/day cocaine HCl group differed significantly from saline controls in ptosis, $p < 0.05$ and gasps/writhes, $p < 0.05$.

No rats in any treatment group displayed any gaps in the skin or large areas of necrotic tissue. Three rats in the saline group, two rats receiving 40 mg/kg/day cocaine HCl, and three rats receiving 60 mg/kg/day cocaine HCl displayed small (<2-mm diameter) areas of necrotic or hardened tissue. Thus, there is little evidence of major tissue damage resulting specifically from cocaine infusion.

EXPERIMENT 2: COCAINE REVERSES COCAINE ABSTINENCE SYNDROME

Method

Methods were identical with Experiment 1 with the following exceptions. The subjects were eight rats, each infused for 7 days with 60 mg/kg cocaine HCl. All rats were observed for abstinence signs at 35 and 37 h after pump removal near the time of peak abstinence signs in Experiment 1). Five minutes prior to the second observation, four rats received 3 mg/kg SC cocaine HCl in saline, while four received saline alone. Each rat's abstinence reversal score was its overall postinjection abstinence signs as a percentage of its preinjection abstinence signs. Thus, 100% would indicate no reduction in signs, while 0% would indicate a complete elimination of abstinence syndrome.

Results

Following saline injection, abstinence signs declined by 4.9% to 95.1 ± 20.7% (mean SEM) of preinjection signs. Following cocaine injection, abstinence signs declined by 83.3% to 17.7 ± 3.5% after cocaine. This difference was significant, $t(6) = 3.68$, $p < 0.01$.

EXPERIMENT 3: TIME COURSE OF COCAINE ABSTINENCE SYNDROME AND ALTERATIONS IN LOCOMOTOR ACTIVITY

Method

Methods were identical with Experiment 1 with the following exceptions. The subjects were 17 rats with weights ranging from 350–430 g. Eight rats were infused for 7 days with 60 mg/kg/day cocaine HCl, while nine rats were infused with saline alone. Each rat was observed for abstinence signs for 15 min on five occasions: 24 h prior to pump implantation, 84 and 156 h following the start of infusion, and 36 and 84 h after pump removal. In this experiment, ptosis was counted no more than once in 30 s (instead of once every minute). This change was made to increase the sensitivity of the withdrawal index, because ptosis was one of the signs that best differentiated the high infusion rate, low infusion rate and control groups (Fig. 3).

Following each observation, each rat was placed in a clear plastic chamber on a Stoelting horizontal activity monitor connected to an IBM Thinkpad computer. The system tallied horizontal activity over 15 min by cumulating the crossing of photoelectric beams placed 2 cm apart. The activity measurements were taken during the last hour of the daily light phase. Each rat's activity score was its activity counts at a given observation as a percentage of its baseline counts (prior to pump implantation).

Results

Figure 3 shows overall abstinence signs as a function of cocaine vs. saline infusion at the five different observation times. Data from the five sequential observations of nine sa-

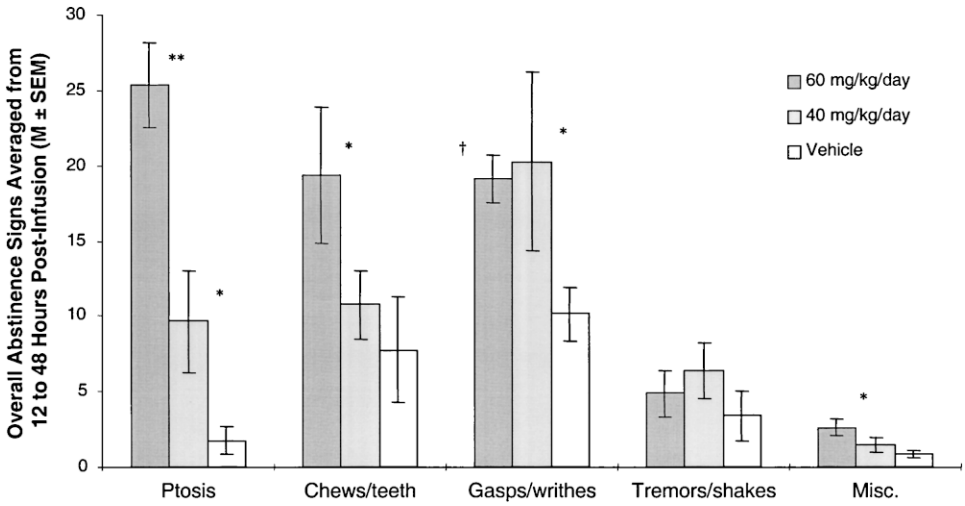

FIG. 2. Individual categories of signs (mean ± SEM) displayed by rats that had received 40 or 60 mg/kg/day cocaine HCl or saline vehicle alone, averaged for each rat over the 12 through 48-h period. $^{**}p < 0.01$, $^{*}p < 0.05$, †$0.05 < p < 0.10$ vs. saline-infused group (Dunnett's test).

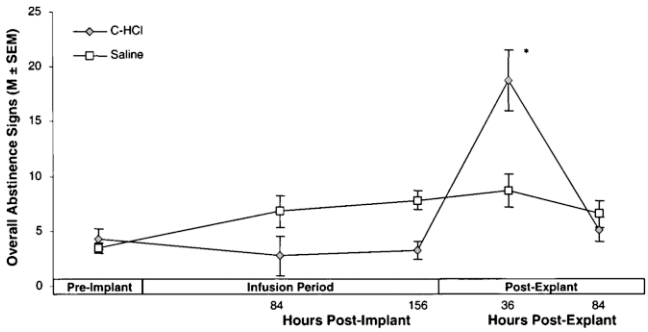

FIG. 3. Overall abstinence signs (mean ± SEM) in rats infused with 60 mg/kg/day cocaine HCl or with saline alone. Rats were observed 12 h before start of infusion (baseline), at 84 and 156 h after the beginning of infusion, and at 36 and 84 h postexplant. *$p < 0.05$ vs. all other groups of scores (Tukey's HSD).

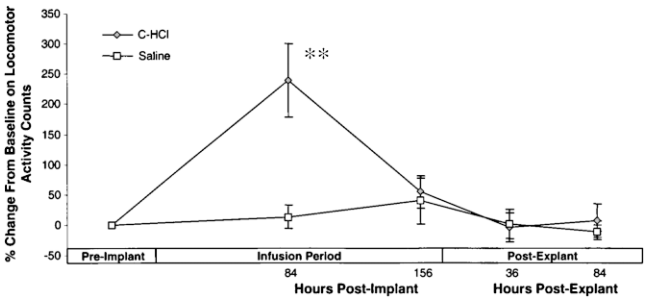

FIG. 4. Activity counts over 15 min as a percentage of preinfusion baseline counts in rats infused with 60 mg/kg/day cocaine HCl or with saline alone. Rats were observed 12 h before start of infusion (baseline), at 3.5 days and 6.5 days (84 h and 156 h) after the beginning of infusion, and 36 and 84 h postexplant. **$p < 0.01$ vs. all other groups of scores (Tukey's HSD).

line-infused and eight cocaine-infused rats was analyzed by two-way ANOVA with one repeated-measures variable. ANOVA revealed: no significant main effect of drug infusion, $F(1, 15) = 0.18$, NS, a significant main effect of time of observation, $F(4, 60) = 13.97$, $p < 0.01$, and a significant interaction effect (drug infusion × time of observation), $F(4,60) = 7.67$, $p < 0.01$. The significant interaction indicated that the effect of cocaine exposure depended on when the rats were observed. Post hoc analysis (Tukey's HSD) revealed that the abstinence scores of cocaine-infused rats at 36 h after pump removal were significantly higher than all other sets of scores, $p < 0.05$. No other differences reached significance.

Figure 4 shows activity (as percentage of baseline) of saline and cocaine-infused rats at two times during the infusion period and two times during the withdrawal period. The cocaine group had an activity surge when measured at 3-1/2 days after the start of infusion, but after 6-2/1 days of infusion their activity was near baseline and almost identical to the saline group. At 36 and 84 h postexplant, both groups displayed similar near-baseline activity levels. Analysis of variance with one repeated measures variable revealed a significant main effect of drug infusion, $F(1,15) = 7.70$, $p < 0.05$, a significant main effect of time of observation, $F(3, 45) = 9.47$, $p < 0.01$, and a significant interaction effect (drug infusion × time of observation), $F(3,45) = 9.98$, $p < 0.01$. Post hoc analysis (Tukey's HSD) revealed that the abstinence scores (as percentage change from baseline) of cocaine-infused rats at 3.5 days of infusion (84 h prior to pump removal) were significantly higher than all other sets of scores, $p < 0.05$. No other differences were significant. In particular, the cocaine and saline groups did not differ significantly on the last day of infusion and during the withdrawal period.

EXPERIMENT 4: THE EFFECT OF DURATION OF COCAINE INFUSION

Method

The methods were the same as in Experiment 3, with the following exceptions. Eight rats were infused with 60 mg/kg/day cocaine HCl for 1-1/2 days (36 h), while eight rats were infused with saline vehicle alone for the same duration. Seven rats were infused with 60 mg/kg/day cocaine HCl for 6-1/2 days (156 h), while seven rats were infused with saline vehicle alone for the same duration. All rats were observed for abstinence signs 36 h after termination of infusion.

Results

Figure 5 shows the overall abstinence signs in all four groups. Two-way ANOVA indicated that the drug effect (cocaine vs. saline) approached significance, $F(3, 26) = 3.80$, $0.05 < p < 0.10$. The duration effect was not significant, $F(3, 26) = 1.75$, NS. The interaction effect (drug × duration) was significant, $F(3, 26) = 5.57$, $p < 0.05$, indicating that the effect of cocaine infusion depends on the duration of infusion. Post hoc analysis (Tukey's HSD), determined that 6-1/2 days of cocaine infusion resulted in significantly more abstinence signs than 6-1/2 days of saline infusion, $p < 0.05$. In contrast, there was no significant difference between the cocaine or saline animals after 1-1/2 days of infusion.

GENERAL DISCUSSION

In four separate experiments, an overall index of cocaine abstinence signs was significantly elevated following termination of continuous subcutaneous cocaine infusion. This model met a number of validity criteria. 1) There were significantly more signs following infusion of cocaine HCl in saline than following similar infusion of saline alone. 2) The occurrence of signs was dose-related (varied with infusion rate). 3) There were significantly more signs 36 h after terminating drug infusion than before infusion, during infusion, or after an extended recovery period. 4) Chronic cocaine infusion was necessary for dependence induction, as indicated by subsequent abstinence syndrome. Infusion for 6-1/2 days, but not 1-1/2 days resulted subsequently in a significant abstinence syndrome. 5) Abstinence signs were largely reversed by injection of 3 mg/kg SC cocaine HCl.

The behavioral signs appear to reflect cocaine abstinence more sensitively than do changes in locomotor activity. Cocaine sharply increased locomotor activity at the midpoint of the infusion period. This effect was greatly reduced by the end of infusion, presumably reflecting the development of cocaine tolerance. During the withdrawal period, locomotor activity levels of both the cocaine and the saline groups remained near baseline levels, with almost no difference between treatment groups. This negative result should be interpreted cautiously in view of data suggesting that locomotor depression in amphetamine withdrawal is much stronger when rats are tested in a novel environment (41). The subjects in the current study were habituated to the test environ-

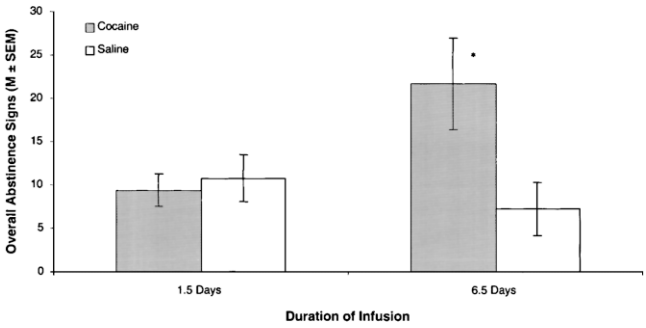

FIG. 5. Overall abstinence signs (mean ± SEM) 36 h following termination of 1.5 or 6.5 days of cocaine HCl or saline infusion. *$p <$ 0.05 vs. 6.5 day saline-infused group (Tukey's HSD).

ment before the onset of cocaine withdrawal. It will be of interest in the future to measure the locomotor activity of cocaine abstinent rats without prior habituation and at differing phases of the daily light cycle.

The behavioral disturbance induced by cocaine withdrawal in the rat is rather prolonged. In Experiment 1, signs were elevated at 12 h postexplant, and remained somewhat elevated for at least 84 h. The time course shown in Figures 1 and 4 is generally consistent with the observed time course of human cocaine withdrawal syndrome (40). Further, development of rat cocaine dependence and subsequent abstinence syndrome required prolonged cocaine exposure. This is consistent with observations that severity of human cocaine abstinence is related to chronicity of use (14). There are, of course, important differences between the laboratory model and human cocaine dependence. In the model, cocaine is administered continuously, while human cocaine users consume the drug in discrete episodes. However, it has been argued (23) that continuous subcutaneous cocaine infusion constitutes a good alternative animal model of some aspects of high-dose cocaine addiction, particularly the "cocaine binge." During a binge, which can last for days or weeks, the user maintains a reasonably sustained plasma cocaine level (23). Opiate narcotic and nicotine users also consume their drugs in discrete episodes, yet continuous infusion models have proved useful in elucidating certain mechanisms of opiate and nicotine dependence and abstinence syndrome (7,17,34).

It is interesting that the general categories of behaviors elevated in cocaine withdrawal are also elevated in morphine (11) and nicotine (30) abstinence syndromes. Rats infused with 40 mg/kg/day cocaine HCl and rats infused with 9 mg/kg/day nicotine bitartrate (30) both displayed abstinence signs in this rank order of occurrence: gasps/writhes > teeth chatter/chews > ptosis > head shakes/tremors > miscellaneous signs. Rats infused with 60 mg/kg/day cocaine HCl displayed a somewhat different rank order of signs: ptosis > teeth chatter/chews > gasps/writhes > head shakes/tremors > miscellaneous signs. The numerous occurrences of ptosis in this treatment group was the most striking difference observed between cocaine abstinence syndrome and nicotine or morphine abstinence syndromes.

Recent evidence (25,28,31,32) suggests that nicotine abstinence signs resemble opiate abstinence signs because of overlapping neurochemical mechanisms: nicotine releases endogenous opioid peptides (4,12,15,20,38,39,43) which in turn, chronically overstimulate opiate receptors, resulting in an opiate dependence-like state. As with nicotine, cocaine exposure of various durations alters the synthesis or amount of various endogenous opioid peptides and opiate receptors (13,19,42,44–46,48,51). In addition, various behavioral effects of cocaine are attenuated by opiate antagonists (2,3,9,18,44,47), suggesting that they may be dependent on endogenous opioid peptide release and opiate receptor stimulation. It remains to be seen whether an endogenous opiate mechanism can account for the resemblance of cocaine abstinence signs to some of those seen in opiate abstinence syndrome. It is hoped that the simple and rapid model of cocaine dependence presented here will be useful for testing this and other hypotheses regarding the underlying mechanism of cocaine dependence and abstinence syndrome. The model might also be useful for preliminary screening of potential therapeutic interventions for managing abstinence syndrome in cocaine users.

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