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Inhibition of cocaine self-administration by fluoxetine or D-fenfluramine combined with phentermine

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Abstract

Instrumental responding for intravenous cocaine in rats at 85% of free-feeding weight was significantly decreased 50% by D-fenfluramine plus phentermine (D-Fen/Phen, 5 mg/kg of each for 1 day). A similar effect was obtained in normal-weight rats self-administering a cocaine — heroin mixture. Treating normal-weight animals with fluoxetine (5 mg/kg) for 4 days also significantly decreased cocaine self-administration by half, and then adding phentermine caused an additional decrease in cocaine intake. Animals that were well trained to self-administer drug did not self-administer intravenous D-Fen/Phen or Flu/Phen. The present results confirm that serotonergic drugs can decrease cocaine, or cocaine/heroin, self-administration in rats, and that phentermine adds to the effect. Based on related research with the same dose of D-Fen/Phen, it is suggested that effectiveness in reducing cocaine reinforcement is due in part to a satiating effect in which dopamine and acetylcholine are released in the nucleus accumbens.

Keywords: Cocaine; Self-administration; D-Fenfluramine; Fenfluramine; Phentermine; Fluoxetine; Speedball; Heroin; Rat

1. Introduction

There has been a long search for treatments that effectively and safely reduce self-administration of addictive drugs such as cocaine. In the rat model, serotonergic drugs have been sometimes shown to reduce intravenous self-administration of cocaine. This has been demonstrated with a serotonin precursor L-tryptophan (Carroll et al., 1990b; McGregor et al., 1993) and the reuptake blocker fluoxetine (Carroll et al., 1990a; Peltier and Schenk, 1993; Richardson and Roberts, 1991); although negative results were also reported (Porrino et al., 1989; Tella, 1995) and clinical efficacy of fluoxetine was questioned (Grabowski et al., 1995). Serotonin depleters and receptor blockers can decrease cocaine seeking under some conditions (Schenk, 2000; Tran-Nguyen et al., 1999). This suggested that serotonergic drugs by themselves are of limited use. The question addressed here is how to make them more effective.

Drugs of abuse act in part on a system that reinforces eating behavior (Di Chiara et al., 1993; Hoebel, 1985; Hoebel and Hernandez, 1990; Hoebel et al., 1999; Wise, 1998). Therefore, researchers have hypothesized that appetite suppressants might help to inhibit drug intake (Rada and Hoebel, 2000b). The combination of D,L-fenfluramine, which is largely serotonergic (Garattini et al., 1986), and phentermine that is partly dopaminergic and noradrenergic, and perhaps indirectly serotonergic as well (Baumann et al., 2000, Mendlin et al., 1999; Rowland et al., 2000) increases extracellular monoamines in various parts of the brain (Balcioglu and Wurtman, 1998; Shoaib et al., 1997) and proved especially potent for appetite reduction and weight loss in therapy for obesity (Weintraub, 1992). The combination also reduced cocaine intake in animals (Glowa et al., 1997; Rothman et al., 1998) and may reduce cocaine-related symptoms in humans (Hitzig, 1993; Kampman et al., 2000), but this combination was reported to have toxic side effects in rats (Harvey and McMaster, 1977; Lew et al., 1997) and humans (Teramae et al., 2000). Other drug combinations and multifunction drugs were then tested in a variety of contexts in attempts to inhibit the appetite for food or drugs, e.g., D-fenfluramine (D-Fen) plus phentermine (Ehrlich et al.,

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1996), fluoxetine plus phentermine (Devlin et al., 2000; Rowland et al., 2000), phentermine alone, which has multiple monoaminergic effects (Baumann et al., 2000; Rothman et al., 2001; Wojnicki et al., 1999) and combination serotonin-norepinephrine reuptake inhibitors (Heal et al., 1999). There is a need to relate these treatments to an underlying neurochemical theory to aid in the development of effective therapy.

During a satiating meal, acetylcholine is released in the nucleus accumbens (Mark et al., 1992). This may contribute to normal satiety by inhibiting incentive motivation or other aspects of reinforcement (Hoebel et al., 1999; Leibowitz and Hoebel, 1998; Rada and Hoebel, 2000b; Rada et al., 2000). Therefore, appetite suppressants that mimic normal satiety by increasing extracellular acetylcholine in the accumbens would theoretically be candidates for drug abuse therapy. The D-isomer of fenfluramine (dexfenfluramine) is interesting as a serotonin uptake blocker, releaser and receptor agonist (Grignaschi et al., 1995; Neill and Cooper, 1989; Samanin et al., 1991). A prior report from this laboratory showed that treatment with D-fenfluramine combined with phentermine (D-Phen/Fen) is one means of increasing the release of acetylcholine in the nucleus accumbens, along with increased dopamine, thus, mimicking these aspects of the satiation process (Rada and Hoebel, 2000b). The purpose of the present study was to use the same dose of D-Fen/Phen that had been shown to release accumbens acetylcholine and dopamine in the feeding study, to determine if this would inhibit cocaine self-administration. Fluoxetine combined with phentermine was also tested. The results with both drug combinations were positive.

2. Method

2.1. Subjects and apparatus

Two groups of male Sprague–Dawley adult rats weighing 300–500 g were housed on a reverse day-night schedule in temperature-controlled facilities. The animals were bred and raised at Princeton University (Experiment 1) or purchased from Taconic (Experiment 2). Each animal was implanted with a jugular vein catheter and trained to self-administer cocaine or a cocaine-heroin mixture in its own Plexiglas chamber equipped for operant conditioning. Each chamber was equipped with a signal light and a lever that delivered 45 mg Noyes food pellets and/or intravenous drug delivery. The intravenous catheter was connected to a syringe pump via an overhead swivel and counterbalanced arm.

2.2. Procedures for training and testing

The experiment was conducted in two parts with male rats ($N=7$ total). Four rats at a reduced body weight were trained with cocaine (Experiment 1a) and three at normal weight with a cocaine-heroin mixture (Experiment 1b). This experimental protocol was approved by the Institutional Review Committee for the use of animal subjects.

2.2.1. Experiment 1a: D-Fen/Phen and cocaine self-administration

Male rats were maintained at 85 ± 5% of normal body weight by daily food restriction and trained to lever press for food pellets on a continuous reinforcement schedule with a 5-s simultaneous cue light. When an animal learned to provide itself with food in this manner, a silastic catheter (Konisberg Instruments) was constructed with two small silicone bumps 3 cm from the beveled end. Rats were anesthetized with xylazine supplemented with ketamine. The catheter was implanted in the jugular vein with 0–0 silk thread (Ethicon) and attached to stainless steel tubing of a connector pedestal (Plastics One, #313-001) and secured to the skull. The catheter attachment point was bolstered with a piece of PE-160 tubing, or in later animals, eliminated by the use of continuous tubing from the top of the connector to the vein. The catheter was then flushed with heparinized saline (33 units/cc). Incisions were treated with Bacitracin ointment and the rat was given intramuscular penicillin (Butler). Three days later the animal was placed in its self-administration chamber and permanently connected to the syringe pump.

Each daily session lasted 4 h in the middle of the dark cycle. A ration of chow was given at the end of the session to maintain 85% body weight. Initially, lever presses triggered a cocaine infusion (0.25 mg/0.1 ml saline/infusion) plus the accustomed food pellet and 5-s light. After 2 days, the food pellet delivery was stopped, and the animal responded for cocaine and cue light. Responses were recorded on a cumulative counter and a strip-chart recorder. Each experimental session began with three priming infusions of cocaine. Following each session, the catheter was flushed with 0.1 cc of heparinized saline to prevent clotting. Rats that pressed at widely fluctuating baseline rates from day to day were excluded from the study. The remaining subjects were tested daily for about a week, during which time their response rate for cocaine stabilized to within 15% for 3 consecutive days. The experimental animals were then given D-fenfluramine (Servier Laboratories) combined with phentermine (Sigma) at a dose of 5 mg/kg of each drug in saline ip, 15 min prior to the start of a 4-h self-administration session, using an ABA design (Day 1: saline; Day 2: D-Fen/Phen; Day 3: saline).

2.2.2. Experiment 1b: D-Fen/Phen and cocaine–heroin self-administration

Male rats were prepared with catheters (Can-Am Research Lab Supplies) exiting between the shoulder blades via a connector attached to the skin and velcro body jacket (Can-Am Res.). Rats in this group were trained to self-administer drug in the same fashion as above, but at a normal body weight. Cocaine was mixed

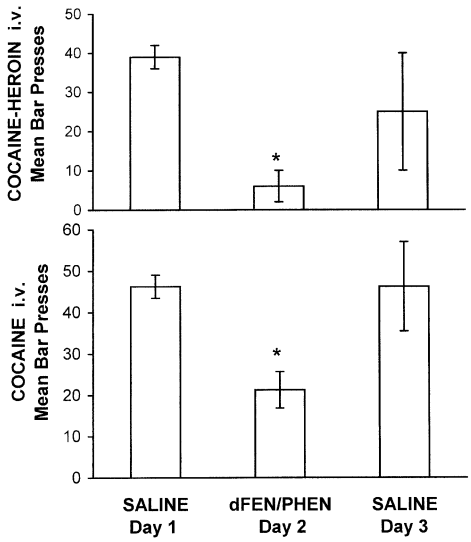

Fig. 1. Rate of lever pressing for intravenous cocaine (lower graph, Experiment 1a) and for a cocaine–heroin mixture (upper graph, Experiment 1b) during 4-h sessions on 3 successive days in an ABA design, with and without intraperitoneal injection of D-fenfluramine combined with phentermine (5 mg/kg of each). D-Fen/Phen significantly reduced self-administration of the drugs of abuse. Asterisks indicate $P < .05$ in the upper graph and $P < .01$ in the lower graph.

with heroin for self-administration (0.18 mg/kg cocaine plus 0.30 mg/kg heroin/0.01ml/2-s infusion iv) for 6 days of training, and then the dose was halved to 0.09 mg/kg cocaine plus 0.15 mg/kg heroin per infusion for 7 more days of training. After 7 days of stable responding for drug during the 4-h daily access period, the 3-day ABA-treatment design was then used as above.

2.2.3. Experiment 2: Flu/Phen and cocaine self-administration

For the Flu/Phen experiment, animals were fed between cocaine tests by allowing them to lever press for Noyes pellets ad libitum. Each response for a food pellet also triggered a 0.2-ml injection of intravenous saline, limited to 3 ml/day, to keep the cannula patent. During the 4-h cocaine access period, lever presses delivered cocaine (as in Exp. 1a) and the 5-s light, but not food. Rats readily distinguished the two conditions, as shown by hovering and rearing at the lever and light during cocaine self-administration instead of traversing the cage to the food magazine. Following a stable baseline, the first 4 days of treatment consisted of intraperitoneal injections of fluoxetine (Eli Lilly, 5 mg/kg) given 15 min prior to cocaine access. On the fifth day, phentermine was added to the fluoxetine (5 mg/kg of each drug in saline ip). The sixth day no drug treatment was given in order to establish that the self-administration system was working properly and cocaine was still a potent reinforcer by itself. If the apparatus or headpiece broke during the experiment, the rat was removed from the study; however, the data up to that time point were retained as valid. Thus, the number of rats for baseline days was 10, and 5 for Day 5 when Flu/Phen was given. Then, two rats were removed to for testing at a later date, leaving three for the recovery test on Day 6.

To determine if rats would self-administer Flu/Phen, two from this study and two more that were well trained to self-administer cocaine *de novo* were offered the Flu/Phen combination (0.25 mg of each drug/0.1 ml/infusion) to self-administer as a substitute for cocaine. Three others from the other experiments were offered D-Fen/Phen, and three, saline.

3. Results

In Experiment 1a, intraperitoneal D-Fen/Phen significantly decreased lever pressing for intravenous cocaine. Rats learned to respond slowly and consistently for cocaine during the 4-h baseline tests when each response turned on a stimulus light and a drug infusion. Systemic injection of D-Fen/Phen decreased total responding about 50% (Fig. 1, bottom graph). In each case, responding returned to baseline levels the next day. Statistical analysis of cocaine lever press rate by ANOVA for four conditions — baseline, saline Day 1, D-Fen/Phen Day 2 and saline Day 3, showed a significant effect of the D-Fen/Phen ($N = 4$; $F(3.12) = 7.12$; $P < .01$). Comparing Day 1 with Day 2, in the ABA design, paired t tests showed cocaine responses with intraperitoneal D-Fen/Phen (mean 21.2, S.E.M. 4.4) were significantly less than when intraperitoneal saline was given as a control (mean 46.2, S.E.M. 2.8). As seen in Fig. 1, responding returned to normal on Day 3 (46.2, S.E.M. 10.8). The 2 saline days (Days 1 and 3) were not significantly different.

In Experiment 1b, rats decreased their response rate for cocaine-heroin from 39 to 5.7 responses per 4-h

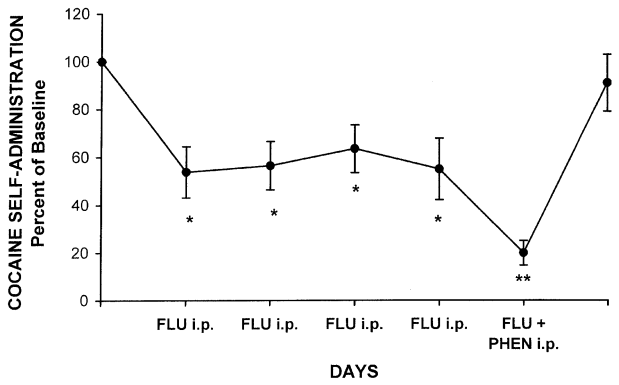

Fig. 2. Daily cocaine self-administration is plotted as a percent of the baseline rate. Self-administration decreased significantly on the 4 successive days when the rats received intraperitoneal fluoxetine. The effect was enhanced the following day when the fluoxetine (5 mg/kg) was combined with phentermine (5 mg/kg). The last day, with no treatment, self-administration returned to baseline (* $P < .05$; ** $P < .01$).

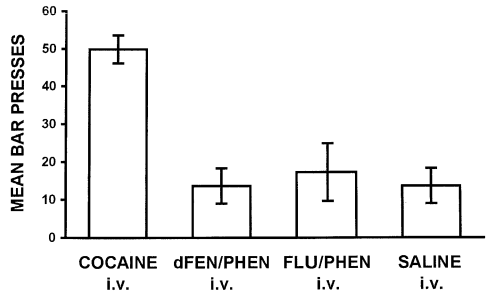

Fig. 3. Rats that were experienced drug self-administrators showed response extinction leading to a significantly lower rate of responding for either D-Fen/Phen, Flu/Phen or normal saline (intravenous) in place of the drug of abuse.

session ($N=3$; $t=5.0$; $P<.05$) on the day they were given intraperitoneal D-Fen/Phen (Fig. 1, top graph). The 2 saline treatment days were not significantly different from each other.

In Experiment 2, fluoxetine alone decreased cocaine response rate on each of 4 days with no difference between days (Fig. 2). The addition of phentermine further decreased responding [$F(6,56)=6.558$; $P<.001$]. When Flu/Phen treatment was removed, cocaine responding returned to baseline levels.

In the substitution tests, rats failed to self-administer D-Fen/Phen or Flu/Phen in place of cocaine. In four animals with a mean cocaine self-administration rate of 50 responses/4 h, the response rate decreased significantly to 17 responses on the day Flu/Phen was delivered intravenously in lieu of cocaine ($N=4$; $t=5.27$; $P<.05$). Similarly, D-Fen/Phen ($N=3$) was not self-administered any more than the operant rate for intravenous saline (Fig. 3).

4. Discussion

Cocaine was a positive reinforcer in these experiments as shown by consistent daily responding during baseline tests. Experiment 1a shows that rats accustomed to responding for intravenous cocaine take about half as much when treated with intraperitoneal D-fenfluramine plus phentermine. This experiment was conducted at 85% of normal body weight, which is known to lower extracellular dopamine in the nucleus accumbens (Pothos et al., 1995a,b) and potentiate cocaine self-administration (Carroll, 1985; De Vry et al., 1989; Glick et al., 1996) and electrical self-stimulation (Cabeza de Vaca and Carr, 1998). Experiments 1b and 2 were conducted at normal body weight, with very similar results. Therefore, body weight does not seem to be a major factor in the present design. The results show that this dose of D-Fen/Phen is effective in curbing cocaine intake in the short term and confirms other studies (Rothman et al., 1998). The dose of D-Fen/Phen that was used will reduce food intake and is exactly the dose that increased extracellular dopamine and acetylcholine in the accumbens in a prior experiment (Rada and Hoebel, 2000b).

Fluoxetine combined with phentermine had a very similar effect to D-Fen/Phen on cocaine self-administration. Fluoxetine was given repeatedly for 4 days, because it was anticipated that the onset of effectiveness might be gradual (Blier and de Montigny, 1994). In fact, the drug was effective in reducing cocaine intake to 60% of baseline beginning on the first day of treatment (see Fig. 2). The rapid, beneficial effect of fluoxetine was similar to that reported by others (Richardson and Roberts, 1991). In the present study, when phentermine was added to fluoxetine in equal proportion, cocaine intake decreased further to 20% of baseline (Fig. 2). This suggests that the combination is more effective that fluoxetine alone. The results are in agreement with other studies showing decreased cocaine responding with serotonergic drug treatment (Peltier and Schenk, 1993), dopamine agonist treatments (Pulvirenti and Koob, 1994) and combination serotonin/dopamine agonist treatments (Glowa et al., 1997).

The first question is whether the pharmacological treatments inhibited cocaine reinforcement, substituted for cocaine, or simply caused malaise or paralysis. The fact that the treatments have been used with humans, as well as in numerous animal studies, helps to answer this question in favor of the reinforcement-inhibition explanation. Fluoxetine, D-Fen and Phen are all appetite suppressants in humans and animals. They are not cocaine substitutes, with the possible exception of phentermine, which is self-administered by rats when they are underweight (Papasava et al., 1985). The present study found no evidence for self-administration of D-Fen/Phen or Flu/Phen at normal body weight. Using activity level to judge malaise, the literature suggests that these drugs, given separately or together, do not impair activity in rats (Carroll et al., 1990a; Richardson and Roberts, 1991) or cause aversive effects in humans (Anchors, 1997). The observation that cocaine self-administration resumed at baseline levels the day after D-Fen/Phen or Flu/Phen treatment suggests there was no residual illness and no aversion conditioning. It has been suggested that some drug therapies enhance the adverse symptoms of cocaine withdrawal, thereby affecting future cocaine intake (McCance, 1997). Drug therapies might also decrease withdrawal symptoms and thereby reduce the need for cocaine. Although such considerations are factors in long-term therapy, they did not enter into the present studies because the drug treatments acted on the first day. The most parsimonious explanation is that D-Fen/Phen somehow satiated the animal for cocaine by inhibiting the reinforcing property of the cocaine without causing compensatory responding for more.

The neurochemical substrates for anorectic drugs provide clues as to why D-Fen/Phen or Flu/Phen might satiate or inhibit cocaine intake. Dopamine, acetylcholine, serotonin and norepinephrine are all strongly implicated. Cocaine and phentermine may act on systems where eating, sexual

behavior and stress can increase extracellular dopamine in the nucleus accumbens (Abercrombie et al., 1989; Baumann et al., 2000; Becker et al., 2000; Hernandez and Hoebel, 1988; Pettit and Justice, 1989; Pfaus et al., 1995; Tanda and Di Chiara, 1998; Westerink et al., 1997; Wise et al., 1995). Extracellular dopamine levels are related to the amount of cocaine or amphetamine self-administered (Ranaldi et al., 1999; Wise et al., 1995) or the schedule of responding for food (Sokolowski et al., 1998). Perhaps, the accumbens dopamine released by phentermine helps alleviate any tendency to compensate with more cocaine (Baumann et al., 2000). On the other hand, dopamine might also act via the hypothalamus where it can function indirectly to inhibit dopamine release in the accumbens reinforcement system (Parada et al., 1990, 1995). The fact that animals self-inject dopamine or psychostimulants into the accumbens shows this is an important locus of dopamine reinforcement (Guerin et al., 1984; Hoebel et al., 1983; McBride et al., 1999). The term reinforcement simply refers to the contingent increase in response rate, i.e., self-administration. Evidence is accumulating to show that dopamine in the accumbens can have incentive properties (Beninger and Miller, 1998; Berridge and Robinson, 1998).

Acetylcholine interneurons in the accumbens may oppose some of dopamine's reinforcement functions, including the incentive to self-administer drugs of abuse. Extracellular acetylcholine may rise when a rat starts to become satiated during a meal (Mark et al., 1992) or when D-Fen/Phen is given systemically (Rada and Hoebel, 2000b). This suggests that the D-Fen/Phen combination used in the present study acted in part by increasing cholinergic functions in the accumbens. A recent report from this laboratory suggests why phentermine might augment the serotonergic effects of D-fenfluramine and fluoxetine. Phentermine at 5 mg/kg released acetylcholine in the nucleus accumbens, and this effect more than doubled when the phentermine was combined with an ineffective 5 mg/kg dose of D-Fen (Rada and Hoebel, 2000b). This supra-additive cholinergic action may inhibit the accumbens reinforcement effects of cocaine. This supposition is based on the correlation between ACh inhibition of feeding (Hoebel et al., 1999) and a possible effect on cocaine intake. In addition to the effect on acetylcholine, Phen or D-Fen/Phen given systemically increased extracellular dopamine (Rada and Hoebel, 2000b). Thus there could be a dopamine substitution effect by which phentermine diminishes the need for cocaine. Self-regulation of cocaine dose in a manner that maintains accumbens dopamine at a high level has been shown by microdialysis (Pettit and Justice, 1989; Wise et al., 1995). In the present case, the animals took less cocaine and less cocaine/heroin when given intraperitoneal D-Fen/Phen at the dose used by Rada et al. (2000b) to increase extracellular dopamine and acetylcholine in the nucleus accumbens. Theoretically, it is the acetylcholine that helps keep the animal from engaging in self-administration.

It is quite clear that the drug therapies used here are not acting by blocking cocaine's dopaminergic effects at the dopamine receptor level. The drugs are not known to have dopamine blocking effects. The D-isomer of fenfluramine is not a dopamine antagonist (Garattini, 1995), but if the combination did act that way for some reason, one might expect to see self-administration increase to compensate for loss of receptor activity (Caine et al., 1995). There was no hint of that occurring.

Phentermine is also a norepinephrine releaser (Rothman et al., 2001). Thus it could act as a multifunctional monoaminergic agonist with actions at α-1 receptors in the hypothalamus that contribute to satiation (Wellman and Davies, 1991) or via the ventral noradrenergic bundle which is necessary for satiation, weight control, amphetamine anorexia and aspects of drug withdrawal (Ahlskog and Hoebel, 1973; Ahlskog et al., 1984; Aston-Jones et al., 1999). It is interesting that the subjective effects of amphetamine derivatives in humans correlate with norepinephrine release in vitro. (Rothman et al., 2001).

Serotonin may also have important roles, such as alleviating the depressed mood and motivation that can contribute to drug abuse (Hoebel et al., 1999; Markou et al., 1998). Fluoxetine, which increases extracellular serotonin, clearly reduces cocaine intake in rats, as shown in this and earlier studies (Carroll et al., 1990a; Peltier and Schenk, 1993; Richardson and Roberts, 1991). Cocaine or dopamine can increase extracellular serotonin (Andrews and Lucki, 2001; Essman et al., 1994; Teneud et al., 1996), which may act in part via 5-HT_2 receptors to help control dopamine release or counteract reduced serotonin function during withdrawal (McMillen et al., 1993; Meert and Clincke, 1992; Parsons et al., 1996). Serotonin's role in satiation is well known, with effects documented extensively in the hypothalamus where, in combination with other neurotransmitters, serotonin can indirectly cause a decrease in accumbens dopamine release while also increasing acetylcholine and causing satiety (Rada et al., 1999). On the other hand, serotonin acting locally in the accumbens decreases acetylcholine release, in part via 5-HT_{1a} receptors, which could be one factor alleviating behavioral depression (Chau et al., 2001; Rada et al., 1993).

Paradoxically cocaine itself has dopamine, acetylcholine, norepinephrine and serotonin agonist effects (Cunningham et al., 1996; Mark et al., 1999; Parsons et al., 1996; Teneud et al., 1996), qualitatively similar to the drugs being used here to treat self-administration. However, substitution for cocaine cannot be the entire explanation for the treatment results, because the animals did not self-administer D-Fen/Phen or Flu/Phen. Thus, we suggest that the markedly reduced drug responding is possibly due in part to the interaction of serotonergic compounds with the phentermine, resulting in a supra-additive release of accumbens acetylcholine (Rada and Hoebel, 2000b). This acetylcholine apparently counteracts dopamine reinforcement of behavior (Hoebel et al., 1999; Mark et al., 1995; Rada and Hoebel,

2000a,b). Perhaps this theory can contribute to the development of useful therapeutics to forestall the development of drug abuse or prevent relapse.

Acknowledgments

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