Extinction Training Regulates Tyrosine Hydroxylase during Withdrawal from Cocaine Self-Administration

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Chronic exposure to drugs of abuse is known to modulate tyrosine hydroxylase (TH) levels in the mesolimbic dopamine system. In this study, 12 d of cocaine self-administration in rats (4 hr/d) reduced TH immunoreactivity by 29% in the nucleus accumbens (NAc) shell, but not core, after a 1 week withdrawal period. In contrast, TH immunoreactivity in the NAc was completely restored in animals that experienced extinction training (4 hr/d) during the same withdrawal period. Extinction training also increased TH levels in the ventral tegmental area (VTA) by 45%, whereas TH was not altered in the VTA by cocaine withdrawal alone. Thus, extinction-induced normalization of NAc TH levels could involve increased TH synthesis, stability, and/or transport from the VTA to the NAc. A similar extinction training regimen failed to alter TH levels in the NAc or VTA of rats trained to self-administer sucrose pellets, indicating that TH regulation in cocaine-trained animals is not a generalized effect of extinction learning per se. Rather, these data suggest that neuroadaptative responses during cocaine withdrawal ultimately are determined by a complex interaction between chronic drug exposure and drug-seeking experience. The ability of extinction training to restore NAc TH levels is hypothesized to accelerate recovery from dopamine depletion and anhedonia during cocaine withdrawal.

Key words: tyrosine hydroxylase; cocaine; dopamine; nucleus accumbens; ventral tegmental area; self-administration; extinction; withdrawal; addiction

The mesolimbic dopamine system is a major neural substrate for the reinforcing effects of psychostimulant drugs, such as cocaine (Wise and Bozarth, 1987; Koob, 1988; Kuhar et al., 1991). Chronic or repeated cocaine administration produces several cellular adaptations in mesolimbic dopamine neurons in the ventral tegmental area (VTA) and in terminal regions, such as the nucleus accumbens (NAc). These neuroadaptations are thought to contribute to addiction-related changes in drug sensitivity, craving, and mood (Koob and Le Moal, 1997; Nestler and Aghajanian, 1997; Self and Nestler, 1998; White and Kalivas, 1998). Cocaine-induced regulation of tyrosine hydroxylase (TH), the rate-limiting enzyme for dopamine synthesis, is well documented in both VTA dopamine neurons and dopamine terminals of the NAc (Trulson et al., 1987; Beitner-Johnson and Nestler, 1991; Sorg et al., 1993; Vrana et al., 1993; Masserano et al., 1996; Todtenkopf et al., 2000). These studies have found that the amount of TH in VTA and NAc increases, decreases, or does not change, depending on the cocaine administration regimen and withdrawal time. Cocaine-induced increases in VTA TH levels may reflect an increase in TH synthesis or an accumulation of TH attributable to impaired axonal transport to terminal regions, resulting in greater somatodendritic dopamine release and autoinhibition of VTA cell firing (Nestler, 1992; Vrana et al., 1993; White and Kalivas, 1998). In contrast, cocaine-induced decreases in TH levels in dopamine terminals may underlie reduced basal dopamine release in the NAc during early withdrawal (Rossetti et al., 1992; Weiss et al., 1992) and could contribute to changes in mood and anhedonia after cocaine self-administration binges (Markou and Koob, 1991; Koob and Le Moal, 1997).

Cocaine-induced regulation of TH and other proteins generally is thought to reflect a neuroadaptive response to the unconditioned pharmacological effects of continued and excessive drug administration. However, most studies use passive, experimenter-delivered cocaine, and several studies have found that self-administered cocaine can have markedly different effects on the brain (Wilson et al., 1994; Dworkin et al., 1995; Hemby et al., 1997; Graziella De Montis et al., 1998; Kuzmin and Johansson, 1999; Mark et al., 1999; Galici et al., 2000). These differences suggest that experiential factors can have a profound influence on the neurobiological response to cocaine and other addictive drugs. Experiential factors also may play a role in modulating neuroadaptive responses during drug withdrawal, although such modulation has not been examined. In this study, we found that

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extinction training modulates TH levels in the NAc and VTA during withdrawal from cocaine self-administration. Extinction training produces a form of behavioral inhibition, whereby conditioned behaviors are masked, rather than reversed, through learning of new contextual relationships (Bouton and Schwartzberg, 1991). We used extinction training to attenuate cocaine-seeking behavior in the context of the environment in which cocaine is self-administered.

MATERIALS AND METHODS

Subjects and surgery. Outbred male Sprague Dawley rats, initially weighing 300–325 gm (Charles River, Kingston, NY), were housed individually in accordance with the *National Institutes of Health Guide for the Care and Use of Laboratory Animals*. To facilitate acquisition of cocaine self-administration, animals were maintained at 85% of original body weight on laboratory chow and trained to press a lever for sucrose pellets until acquisition criteria was achieved (100 sucrose pellets for 3 consecutive test days). Animals were promptly returned to their home cages after completing each training session to prevent exposure to extinction conditions. After lever-press training, animals were fed *ad libitum* and surgically implanted with a chronic indwelling jugular catheter composed of SILASTIC tubing (Green Rubber, Woburn, MA) as described previously (Sutton et al., 2000). The jugular catheter passed subcutaneously to exit the back through 22 gauge cannulas (Plastics One, Roanoke, VA), embedded in cranioplastic cement, and secured with Marlex surgical mesh (Bard Inc., Cranston, RI). Animals received a prophylactic injection of penicillin (60,000 IU, i.m.) and were allowed at least 4 d to recover before cocaine self-administration training. Catheters were flushed daily with 0.2 ml of heparinized (20 IU/ml) bacteriostatic saline containing gentamycin sulfate (0.33 mg/ml). Each group of cocaine-trained animals was paired with age- and group-matched control animals for biochemical comparisons. Control animals received identical lever-press training for sucrose pellets but remained in their home cages throughout the rest of the experiment. Another experimental group continued sucrose training for12 d while maintained at 85% initial body weight as described below.

Apparatus. Cocaine, sucrose, and extinction training were conducted in operant test chambers (Coulbourn Instruments, Lehigh, PA) that were contextually distinct from the animal's home cage and located in a different room. Each chamber was enclosed in a Styrofoam-encased unit and equipped with an infusion pump assembly consisting of a Razel Model A pump (Stamford, CT) and a 20 ml glass syringe connected to a fluid swivel (Stoelting, Wood Dale, IL) by Teflon tubing. Tygon tubing connected the swivel to the animal's catheter assembly and was enclosed by a metal spring. Each operant chamber contained two levers (4 × 2 cm, located 2 cm off the floor). During self-administration training, a single 20 gm lever-press response on the active lever delivered an intravenous infusion of cocaine (1.0 mg/kg per 0.1 ml infusion) over a 10 sec infusion interval. A time-out period was imposed for the 10 sec infusion plus an additional 5 sec, during which the house light was extinguished and responding produced no programmed consequences. Illumination of the house light signaled the end of the 15 sec infusion–time-out period. Under all circumstances, responding at the inactive lever produced no programmed consequence. During sucrose training, a single lever-press response on the active lever delivered a 45 mg sucrose pellet (distinct from lab chow) to a pellet trough located between the levers.

Procedures. Animals were trained to respond for cocaine infusions or sucrose pellets in 12 daily 4 hr test sessions (6 d/week) during their dark cycle. During the second week of cocaine self-administration, animals were given one to two priming injections to ensure initiation of self-administration early in the test session. After the 12 d training phase, cocaine-trained animals were assigned to one of three groups such that the average level of cocaine intake was similar over the final 6 d of cocaine training. One group was killed 12–16 hr after the last self-administration session (1 d withdrawal). A second group of animals remained in their home cage for 1 week until they were killed (1 week withdrawal). A third group returned to the test chambers for extinction training in six daily 4 hr sessions (1 week extinction). A final group of sucrose pellet-trained animals returned to the test chambers for similar extinction training in six daily 4 hr sessions [1 week extinction (sucrose)]. During sucrose training, animals were fed immediately after each training session enough lab chow to maintain 85% of initial body weight, as measured before the next training session; sucrose-trained animals were fed *ad libitum* throughout extinction training and before being killed. During all extinction training procedures, responding on the previously active lever was recorded but had no programmed consequences. Extinction-trained animals were killed 12–16 hr after the last extinction test session. Age- and batch-matched control animals (untreated controls) were killed at the same time as the experimental groups. Each experimental group is composed of animals tested and killed on at least two separate occasions.

Tissue dissection–Western blot procedures. Rats were removed from their home cages and immediately decapitated in a separate room; the brains were rapidly dissected and chilled in ice-cold physiological buffer (in mM: 5 KCl, 126 NaCl, 1.25 NaH_2PO_4, 10 D-glucose, 25 $NaHCO_3$, 2 $CaCl_2$, and 2 $MgSO_4$, pH 7.4). NAc core samples were obtained with a 14 gauge punch from chilled coronal brain slices (0.7–2.2 mm anterior to bregma; Paxinos and Watson, 1998) and immediately frozen and stored at −80°C. Half-moon-shaped NAc shell samples were obtained with a 12 gauge punch of the remaining ventromedial shell tissue. VTA samples were obtained with a 16 gauge punch from coronal brain slices from 5.0–6.3 mm posterior to bregma.

Tissue samples were homogenized by sonication in 350 (NAc) or 150 (VTA) μl of 1% SDS. Protein concentrations were determined (Lowry et al., 1951), and10 μg of protein per sample was subjected to SDS-PAGE (7.5% acrylamide–0.12% bisacrylamide), followed by electrophoretic transfer to nitrocellulose (Bio-Rad, Hercules, CA). TH was immunolabeled with rabbit anti-TH (1:5000; Chemicon, Temecula, CA) incubated overnight at 4°C in blocking buffer consisting of 5% nonfat dried milk powder in PBST (10 mM sodium phosphate, pH 7.4, 0.9% NaCl, and 0.1% Tween 20). After incubation with the primary antibody, blots were washed with blocking buffer and incubated for 2 hr at 20°C with horseradish peroxidase-conjugated goat anti-rabbit IgG (1:2000; Chemicon) in PBST. The blots were washed again in PBST, and immunoreactivity visualized using enhanced chemiluminescence for peroxidase labeling (NEN, Boston, MA). TH immunoreactivity was quantified by densitometric analysis using NIH Image 1.57 (National Institute of Health, Bethesda, MD). TH immunoreactivity was linear over a fourfold range of tissue concentrations under these conditions.

Data analysis. Each gel contained five to eight control samples alternating with samples from experimental animals. To normalize data from different gels, TH immunoreactivity for each control and experimental sample was expressed as a percentage of the mean control value for that particular gel. For statistical analysis, age- and batch-matched control values were pooled into a single group and compared with the three cocaine-trained groups with one-way ANOVA. *Post hoc* comparisons were made among control and cocaine-trained groups with Fisher's tests. TH immunoreactivity in tissue from sucrose-trained animals was compared with their age- and batch-matched control group by Student's *t* tests. Two VTA samples from the 1 week extinction group were discarded *pre hoc* because of dissection abnormalities.

RESULTS

Cocaine-trained groups self-administered similar amounts of cocaine during the last six self-administration training days (Fig. 1, *left*). Mean daily cocaine intake (days 7–12) averaged 39.8 ± 4.1 mg/kg for the 1 d withdrawal group, 36.7 ± 3.5 mg/kg for the 1 week withdrawal group, and 33.5 ± 2.32 mg/kg for the 1 week extinction group. Sucrose-trained animals reached asymptotic response rates within 3 d of training and typically consumed all 100 sucrose pellets within the first 30 min of each subsequent training session. During extinction training, non-reinforced responding on the previously active lever declined to similar levels in both cocaine- and sucrose-trained groups after six daily training sessions (Fig. 1, *right*). Sucrose-trained animals exhibited relatively higher response rates during the initial extinction test when compared with cocaine-trained animals, consistent with previous studies (Tran-Nguyen et al., 1999).

Western blots of NAc core and shell tissue contained a single TH-immunoreactive band (Fig. 2) migrating at ~57 kDa. TH immunoreactivity in NAc shell tissue, but not core, differed among cocaine-trained and untreated control groups ($F_{(3,55)} = 3.669$; $p = 0.018$). After 1 d of withdrawal from cocaine self-administration, a modest 17% reduction in TH levels was found

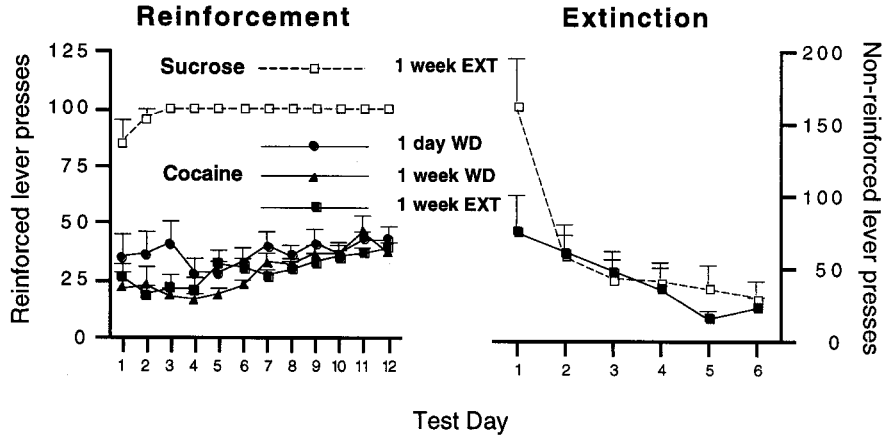

Figure 1. Acquisition and extinction of reinforced lever-press responding for intravenous cocaine injections (1.0 mg/kg per injection) or sucrose pellets (maximum of 100) in daily 4 hr test sessions. Cocaine-trained animals were divided into three groups and killed at 1 d withdrawal (*WD*; $n = 9$), 1 week withdrawal in their home cages ($n = 8$), and after 1 week of extinction training for 4 hr/d in the self-administration test chambers (*EXT*; $n = 14$). Sucrose-trained animals ($n = 10$) were killed after a similar 1 week extinction training regimen. The mean ± SEM number of reinforced lever-press responses is shown on the *left*, and non-reinforced lever press responses during extinction training are shown on the *right*.

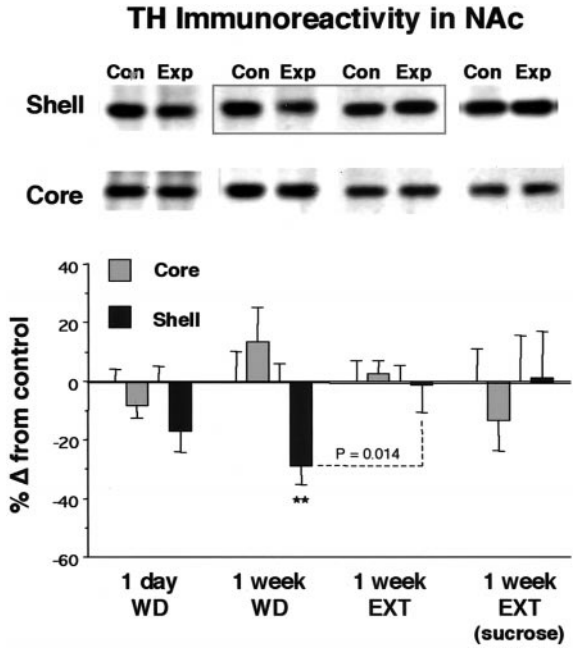

Figure 2. Effects of chronic cocaine self-administration, withdrawal (*WD*), and extinction training (*EXT*) on TH immunoreactivity in NAc core and shell tissue. The effect of extinction training in sucrose-trained animals on TH immunoreactivity also is shown. Data for experimental groups ($n = 8$–14) are expressed as the mean ± SEM percent change from untreated age- and group-matched control tissue ($n = 8$–18). Representative Western blots illustrating regulation of TH immunoreactivity are shown for untreated control (*Con*) and experimental (*Exp*) tissue *above* the graphical data for each group. *Asterisks* indicate a significant difference when compared with pooled untreated control tissue (**$p < 0.01$), and the *dotted line* compares 1 week withdrawal to 1 week extinction training by Fisher's test.

in NAc shell tissue when compared with untreated controls ($p = 0.072$). After 1 week of withdrawal from cocaine, levels of TH immunoreactivity in the NAc shell further decreased to 71% of untreated control values ($p = 0.005$). However, in animals that experienced extinction training for 4 hr/d during the 1 week withdrawal period, TH immunoreactivity in NAc shell tissue was similar (99%) to control values but was increased by 38% relative to animals that remained in their home cages during withdrawal ($p = 0.014$). These data indicate that extinction training reversed decreases in TH immunoreactivity in the NAc shell during withdrawal from cocaine self-administration. A similar extinction-training regimen failed to alter TH levels in NAc tissue from sucrose-reinforced animals when compared with untreated controls.

Regulation of TH immunoreactivity in NAc dopamine terminals could result from altered TH regulation in VTA dopamine cell bodies, and TH levels in VTA tissue differed among cocaine-trained and untreated control groups ($F_{(3,78)} = 2.991$; $p = 0.036$). However, Figure 3 shows that TH immunoreactivity in the VTA was unchanged after both 1 d and 1 week withdrawal from cocaine self-administration, despite lower TH levels in the NAc shell from the same animals. In contrast, animals that experienced extinction training during cocaine withdrawal show a marked 45% increase in TH immunoreactivity in the VTA relative to untreated controls ($p = 0.007$) and a 60% increase when compared with animals that remained in their home cages during withdrawal ($p = 0.016$). Thus, extinction training essentially increased the amount of TH in both the VTA and NAc when compared with animals withdrawn from cocaine without extinction training. Extinction training failed to alter TH immunoreactivity in VTA tissue from sucrose-trained animals.

DISCUSSION

This study examined the effects of chronic cocaine self-administration, withdrawal, and extinction training on regulation of TH immunoreactivity in NAc and VTA tissue. We found that 12 d of 4 hr daily access to cocaine self-administration decreases TH immunoreactivity in NAc shell, but not core, and this reduction persists and intensifies for at least 1 week into withdrawal. Using immunohistological methods, a previous study found a similar reduction in TH immunolabeling of dopamine axons and terminals in the NAc during cocaine withdrawal (Trulson et al., 1987), although core–shell differences were not delineated. A selective reduction in the amount of TH in the NAc shell could explain recent findings in which tolerance develops to cocaine-induced dopamine release in the shell, but not in the core, after repeated cocaine administration (Cadoni et al., 2000). However, other studies have found that repeated cocaine administration

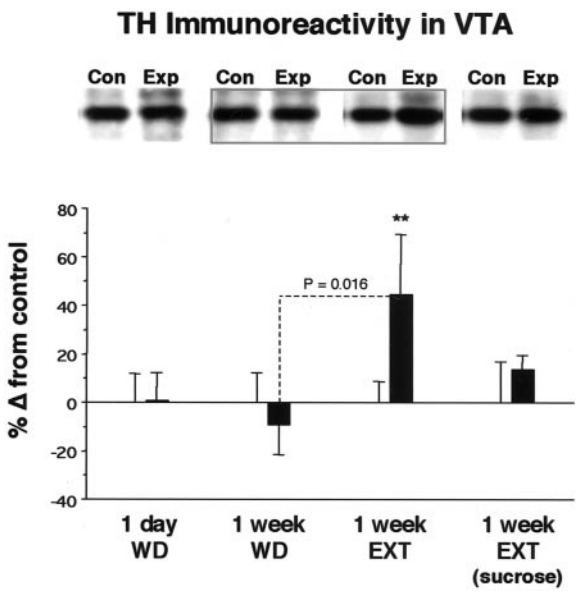

Figure 3. Effects of chronic cocaine self-administration, withdrawal (*WD*), and extinction training (*EXT*) on TH immunoreactivity in VTA tissue. The effect of extinction training in sucrose-trained animals on TH immunoreactivity also is shown. Data for experimental groups ($n = 8–12$) are expressed as the mean ± SEM percent change from untreated age- and group-matched control tissue ($n = 7–21$). Representative Western blots illustrating regulation of TH immunoreactivity are shown for untreated control (*Con*) and experimental (*Exp*) tissue *above* the graphical data for each group. *Asterisks* indicate a significant difference when compared with pooled untreated control tissue (**$p < 0.01$), and the *dotted line* compares 1 week withdrawal with 1 week extinction training by Fisher's test.

produces increases or has no effect on NAc TH levels using Western blots or enzymatic activity (Beitner-Johnson and Nestler, 1991; Sorg et al., 1993; Vrana et al., 1993; Todtenkopf et al., 2000). These discrepant findings may reflect different routes of cocaine administration, treatment duration, and withdrawal times.

It also is possible that self-administered cocaine regulates TH differently from passive administration, although passive administration was not compared in this study. In this regard, we found that a similar regimen of heroin administration (6 hr/d) for 18 d also reduces NAc TH levels, whether heroin is self-administered or passively administered under identical schedules (Self et al., 1995). In addition to cocaine and heroin, chronic ethanol self-administration decreases TH levels in the NAc (Ortiz et al., 1995). Thus, chronic self-administration of a variety of addictive drugs reduces the amount of TH in NAc under conditions of moderate to high daily access. This alteration could contribute to reduced dopamine synthesis and release during the early stages of drug withdrawal after long-term or binge-like drug administration (Brock et al., 1990; Pothos et al., 1991; Rossetti et al., 1992; Weiss et al., 1992; Diana et al., 1993). In contrast, more limited access to self-administered amphetamine, or repeated single injection regimens of cocaine, are both associate with enhanced drug-induced dopamine release in the NAc at later withdrawal times (Kalivas and Duffy, 1993; Lorrain et al., 2000).

In the present study, TH immunoreactivity in the VTA is not altered after 1 d or 1 week withdrawal from cocaine self-administration, whereas previous studies have found increases, decreases, or no effect on TH when cocaine is administered repeatedly by bolus intraperitoneal injections (Trulson et al., 1987; Beitner-Johnson and Nestler, 1991; Sorg et al., 1993). In these latter studies, increases in VTA TH levels primarily are associated with early withdrawal times, whereas decreases and no effect are associated with late withdrawal times. Together, these results suggest that cocaine-induced regulation of TH levels in the VTA is highly dependent on the treatment regimen used. However, our results arguably model human drug self-administration patterns more accurately than bolus intraperitoneal injections, because animals are exposed to a relatively higher daily dose of cocaine, delivered by an intravenous route, and self-regulated over several hours. Given that chronic heroin and ethanol self-administration does increase TH levels in the VTA (Ortiz et al., 1995; Self et al., 1995), perhaps even longer access to cocaine self-administration is required to regulate TH levels in the VTA. In any event, the present findings suggest that cocaine- or withdrawal-induced reductions in NAc TH levels apparently can occur independent of TH regulation in the VTA.

Remarkably, extinction training during withdrawal completely restored TH to normal levels in the NAc shell and increased TH levels in the VTA. In both cases, this regulation represents an increase in TH immunoreactivity relative to animals that remained in their home cages during withdrawal. Restoration of NAc TH levels could result from either an increase in TH synthesis or a decrease in degradation in the VTA, leading to greater transport to dopamine terminals in the NAc. However, other local regulatory mechanisms, such as stabilization of TH in NAc dopamine terminals, cannot be ruled out.

Extinction training failed to alter TH levels in sucrose-trained animals, despite the fact that responding extinguished to a similar extent in both sucrose- and cocaine-trained animals. Higher response rates during extinction training in sucrose- relative to cocaine-trained animals is not attributable to deprivation state during extinction, because animals were fed *ad libitum* during the extinction training phase. Instead, higher extinction responding in sucrose-trained animals probably reflects a greater incentive motivational value of sucrose rewards relative to cocaine, which is enhanced by food deprivation during the sucrose-training phase of the experiment (Balleine and Dickinson, 1998). Thus, failure to find alterations in TH in sucrose-trained animals probably is not attributable to lower reinforcing efficacy of sucrose relative to cocaine rewards. Moreover, these data indicate that regulation of TH by extinction training in cocaine-trained animals cannot reflect a generalized effect of the training procedure itself or a neuroadaptive response to extinction learning per se. Rather, this regulation represents a complex interaction between the neuroadaptive response to chronic cocaine exposure and nonreinforced drug-seeking experience during withdrawal. Although the nature of this interaction is not fully understood, it appears that extinction of cocaine-seeking behavior, when performed in the context of the environment in which cocaine is self-administered, can alter the neurobiological consequence of withdrawal from chronic cocaine self-administration.

Modern incentive motivational theories (Bindra, 1974) posit that instrumental behavior is dependent on learned Pavlovian associations, whereby the presence of drug-related contextual cues is required to elicit instrumental drug-seeking responses. Therefore, it is possible that extinction-induced neural alterations are context-dependent and require that extinction training occur in the context of the drug-taking environment. However, further investigation is needed to determine whether extinction of drug-related contextual associations alone is sufficient for modulation

of TH or whether extinction of instrumental responding is necessary.

In humans, extinction training procedures are used to attenuate conditioned responses to cocaine, including accelerated heart rate, anxiety, and craving (O'Brien et al., 1990; Foltin and Haney, 2000), although their effectiveness in preventing relapse remains unknown. The present study found that extinction training restores TH in the NAc shell of rats during withdrawal from cocaine self-administration. Given that TH is the rate-limiting enzyme for dopamine synthesis, one possibility is that extinction training could help to normalize dopamine depletion and thereby reverse anhedonia and depression during cocaine withdrawal (Markou and Koob, 1991; Koob and Le Moal, 1997). In addition, these results demonstrate that extinction-experienced animals differ from a neurobiological standpoint when compared with animals in cocaine withdrawal alone, which may have profound implications for the validity of certain animal models of drug craving and relapse. For example, extinction-induced changes could alter the subsequent ability of stimuli to trigger relapse to drug-seeking behavior and thus inaccurately model behavioral states in addicted people who have never experienced extinction training. Finally, although it is widely believed that most neuroadaptations to addictive drugs result from repeated and excessive exposure to their unconditioned pharmacological effects, this study underscores the important contribution of experiential factors in ultimately determining brain responses to chronic drug self-administration.

REFERENCES

Balleine BW, Dickinson A (1998) Goal-directed instrumental action: contingency and incentive learning and their cortical substrates. Neuropharmacology 37:407–419.

Beitner-Johnson D, Nestler EJ (1991) Morphine and cocaine exert common chronic actions on tyrosine hydroxylase in dopaminergic brain reward regions. J Neurochem 57:344–347.

Bindra D (1974) A motivational view if learning, performance, and behavior modification. Psychol Rev 81:199–213.

Bouton ME, Schwartzberg D (1991) Sources of relapse after extinction in pavlovian and instrumental learning. Clin Psychol Rev 11:123–140.

Brock JW, Ng JP, Justice Jr JB (1990) Effect of chronic cocaine on dopamine synthesis in the nucleus accumbens as determined by microdialysis perfusion with NSD-1015. Neurosci Lett 117:234–239.

Cadoni C, Solinas M, Di Chiara G (2000) Psychostimulant sensitization: differential changes in accumbal shell and core dopamine. Eur J Pharmacol 388:69–76.

Diana M, Pistis M, Carboni S, Gessa G, Rossetti ZL (1993) Profound decrement of mesolimbic dopaminergic neuronal activity during ethanol withdrawal syndrome in rats: electrophysiological and biochemical evidence. Proc Natl Acad Sci USA 90:7966–7969.

Dworkin SI, Co C, Smith JE (1995) Rat brain neurotransmitter turnover rates altered during withdrawal from chronic cocaine administration. Brain Res 682:116–126.

Foltin RW, Haney M (2000) Conditioned effects of environmental stimuli paired with smoked cocaine in humans. Psychopharmacology 149:24–33.

Galici R, Pechnick RN, Poland RE, France CP (2000) Comparison of noncontingent versus contingent cocaine administration on plasma corticosterone levels in rats. Eur J Pharmacol 387:59–62.

Graziella De Montis M, Co C, Dworkin SI, Smith JE (1998) Modifications of dopamine D1 receptor complex in rats self-administering cocaine. Eur J Pharmacol 362:9–15.

Hemby SE, Co C, Koves TR, Smith JE, Dworkin SI (1997) Differences in extracellular dopamine concentrations in the nucleus accumbens during response-dependent and response-independent cocaine administration in the rat. Psychopharmacology 133:7–16.

Kalivas PW, Duffy P (1993) Time course of extracellular dopamine and behavioral sensitization to cocaine. I. Dopamine axon terminals. J Neurosci 13:266–275.

Koob GF (1988) Separate neurochemical substrates for cocaine and heroin reinforcement. In: Quantitative analysis of behavior: biological determinants of reinforcement and memory (Commons ML, Church RM, Stellar JR, Wagner AR, eds), pp 139–156. Hillsdale, NJ: Erlbaum.

Koob GF, Le Moal M (1997) Drug abuse: hedonic homeostatic dysregulation. Science 278:52–58.

Kuhar MJ, Ritz MC, Boja JW (1991) The dopamine hypothesis of the reinforcing properties of cocaine. Trends Neurosci 14:299–302.

Kuzmin A, Johansson B (1999) Expression of c-fos, NGFI-A and secretogranin II mRNA in brain regions during initiation of cocaine self-administration in mice. Eur J Neurosci 11:3694–3700.

Lorrain DS, Arnold GM, Vezina P (2000) Previous exposure to amphetamine increases incentive to obtain the drug: long-lasting effects revealed by the progressive ratio schedule. Behav Brain Res 107:9–19.

Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:265–275.

Mark GP, Hajnal A, Kinney AE, Keys AS (1999) Self-administration of cocaine increases the release of acetylcholine to a greater extent than response-independent cocaine in the nucleus accumbens of rats. Psychopharmacology 143:47–53.

Markou A, Koob GF (1991) Postcocaine anhedonia. An animal model of cocaine withdrawal. Neuropsychopharmacology 4:17–26.

Masserano JM, Baker I, Natsukari N, Wyatt RJ (1996) Chronic cocaine administration increases tyrosine hydroxylase activity in the ventral tegmental area through glutaminergic- and dopaminergic D2-receptor mechanisms. Neurosci Lett 217:73–76.

Nestler EJ (1992) Molecular mechanisms of drug addiction. J Neurosci 12:2439–2450.

Nestler EJ, Aghajanian GK (1997) Molecular and cellular basis of addiction. Science 278:58–63.

O'Brien CP, Childress AR, McLellan T, Ehrman R (1990) Integrating systemic cue exposure with standard treatment in recovering drug dependent patients. Addict Behav 15:355–365.

Ortiz J, Fitzgerald LW, Charlton M, Lane S, Trevisan L, Guitart X, Shoemaker W, Duman RS, Nestler EJ (1995) Biochemical actions of chronic ethanol exposure in the mesolimbic dopamine system. Synapse 21:289–298.

Paxinos G, Watson GC (1998) The rat brain in stereotaxic coordinates, Ed 4. New York: Academic.

Pothos E, Rada P, Mark GP, Hoebel BG (1991) Dopamine microdialysis in the nucleus accumbens during acute and chronic morphine, naloxone-precipitated withdrawal and clonidine treatment. Brain Res 566:348–350.

Rossetti ZL, Hmaidan Y, Gessa GL (1992) Marked inhibition of mesolimbic dopamine release: a common feature of ethanol, morphine, cocaine and amphetamine abstinence in rats. Eur J Pharmacol 221:227–234.

Self DW, Nestler EJ (1998) Relapse to drug seeking: neural and molecular mechanisms. Drug Alcohol Depend 51:49–60.

Self DW, McClenahan AW, Beitner-Johnson D, Terwilliger RZ, Nestler EJ (1995) Biochemical adaptations in the mesolimbic dopamine system in response to heroin self-administration. Synapse 21:312–318.

Sorg BA, Chen S-Y, Kalivas PW (1993) Time course of tyrosine hydroxylase expression after behavioral sensitization to cocaine. J Phamacol Exp Ther 266:424–430.

Sutton MA, Karanian DA, Self DW (2000) Factors that determine a propensity for cocaine-seeking behavior during abstinence in rats. Neuropsychopharmacology 22:626–641.

Todtenkopf MS, De Leon KR, Stellar JR (2000) Repeated cocaine treatment alters tyrosine hydroxylase in the rat nucleus accumbens. Brain Res Bull 52:407–411.

Tran-Nguyen LT, Baker DA, Grote KA, Solano J, Neisewander JL (1999) Serotonin depletion attenuates cocaine-seeking behavior in rats. Psychopharmacology 146:60–66.

Trulson ME, Joe JC, Babb S, Raese JD (1987) Chronic cocaine administration depletes tyrosine hydroxylase immunoreactivity in the mesolimbic dopamine system in rat brain: quantitative light microscopic studies. Brain Res Bull 19:39–45.

Vrana SL, Vrana KE, Koves TR, Smith JE, Dworkin SI (1993) Chronic cocaine administration increases CNS tyrosine hydroxylase enzyme activity and mRNA levels and tryptophan hydroxylase enzyme activity levels. J Neurochem 61:2262–2268.

Weiss F, Markou A, Lorang MT, Koob GF (1992) Basal extracellular dopamine levels in the nucleus accumbens are decreased during cocaine withdrawal after unlimited-access self-administration. Brain Res 593:314–318.

White FJ, Kalivas PW (1998) Neuroadaptations involved in amphetamine and cocaine addiction. Drug Alcohol Depend 51:141–153.

Wilson JM, Nobrega JN, Corrigall WA, Coen KM, Shannak K, Kish SJ (1994) Amygdala dopamine levels are markedly elevated after self- but not passive-administration of cocaine. Brain Res 668:39–45.

Wise RA, Bozarth MA (1987) A psychomotor stimulant theory of addiction. Psychol Rev 94:469–492.