Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 5880–5884, June 1995
Neurobiology

Decreased brain reward produced by ethanol withdrawal

(addiction/dependence/alcoholism/neuroadaptation)

GERY SCHULTEIS*, ATHINA MARKOU, MAURY COLE, AND GEORGE F. KOOB

Department of Neuropharmacology, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, CA 92037

Communicated by Floyd E. Bloom, The Scripps Research Institute, La Jolla, CA, March 21, 1995

ABSTRACT **Abstinence from chronic administration of various drugs of abuse such as ethanol, opiates, and psychostimulants results in withdrawal syndromes largely unique to each drug class. However, one symptom that appears common to these withdrawal syndromes in humans is a negative affective/motivational state. Prior work in rodents has shown that elevations in intracranial self-stimulation (ICSS) reward thresholds provide a quantitative index that serves as a model for the negative affective state during withdrawal from psychostimulants and opiates. The current study sought to determine whether ICSS threshold elevations also accompany abstinence from chronic ethanol exposure sufficient to induce physical dependence. Rats prepared with stimulating electrodes in the lateral hypothalamus were trained in a discrete-trial current-intensity ICSS threshold procedure; subsequently they were subjected to chronic ethanol administration in ethanol vapor chambers (average blood alcohol level of 197 mg/dl). A time-dependent elevation in ICSS thresholds was observed following removal from the ethanol, but not the control, chambers. Thresholds were significantly elevated for 48 hr after cessation of ethanol exposure, with peak elevations observed at 6–8 hr. Blood alcohol levels were directly correlated with the magnitude of peak threshold elevation. Ratings of traditional overt signs of withdrawal showed a similar time course of expression and resolution. The results suggest that decreased function of reward systems (elevations in reward thresholds) is a common element of withdrawal from chronic administration of several diverse classes of abused drugs.**

Withdrawal from sedative–hypnotic drugs such as ethanol is characterized by a state of central nervous system (CNS) hyperexcitability that can result, in varying degrees of severity, in profound physiological disturbances such as tremors, hyperthermia, seizures, rigidity, hyperreflexia, and hallucinations and delirium (1–3). This constellation of withdrawal signs is largely unique to the sedative–hypnotics, and withdrawal from this class of drugs can be life-threatening.

Other classes of drugs, such as opiates and psychostimulants, are associated with different withdrawal syndromes. The opiate withdrawal syndrome, which has been described as an intense "flu-like state," includes symptoms such as lacrimation, rhinorrhea, sweating, dilated pupils, gooseflesh, intestinal spasm, diarrhea, hyperthermia, anorexia/weight loss, and muscle spasms (2, 4). Withdrawal from chronic abuse of psychostimulants such as cocaine and amphetamines is associated with relatively minor somatic disturbances, including fatigue and suppressed heart rate, and is primarily characterized by more affective or emotional signs such as depression, dysphoria, and anxiety (2, 5). These differences in withdrawal symptomatology are not unexpected given the vastly different pharmacodynamic mechanisms of action of sedative/hypnotic, opiate, and psychomotor stimulant drugs and the unique neuroanatomical localization of receptors with which these different drugs interact. However, there is some clinical evidence that chronic dependence and withdrawal from all three of these classes of abused drugs may share common symptomatology in the form of affective or emotional disturbances, such as irritability, restlessness, anxiety, and mood disturbances such as dysphoria, depression, and anhedonia (1, 2, 4–12). It has been shown in studies with rodents that ethanol, opiate, and psychostimulant withdrawal appear to produce a similar anxiety-like state (13).

Furthermore, despite their distinct pharmacodynamic profiles, ethanol, opiates, and psychostimulants all share reinforcing or rewarding properties upon acute administration, which appear to involve activation of common reward circuits in the brain, including most notably the nucleus accumbens in the basal forebrain and its afferent connections from ventral midbrain (i.e., the ventral tegmental area) and limbic portions of the CNS (14–16).

The symptoms of drug dependence have long been considered from the perspective of neuroadaptation theories. One such theory postulates that all positive reinforcers, including drugs of abuse, produce positive affective responses in the CNS that are opposed by negative affective responses as a natural consequence of an organism's propensity to maintain affective homeostasis (17–19). These negative affective consequences, which are hypothesized to be the consequence of neuroadaptations within the brain reward circuitry (17, 18), may upon termination of drug administration express themselves as a drug-opposite withdrawal response. Accordingly, one might predict that such negative affective consequences of drug withdrawal are common across multiple classes of drugs which share the common property of activating brain reward systems despite diverse pharmacodynamic mechanisms of action.

To that end, it has been shown that acute administration of opiates and psychostimulants, such as cocaine and amphetamines, lower intracranial self-stimulation (ICSS) reward thresholds (20–23), generally considered to be an index of the rewarding properties of these drugs. By contrast, withdrawal from chronic administration of these drugs results in an elevation of ICSS thresholds, which is considered an index of a negative affective motivational state and is opposite to the effect produced by acute administration (24–27).

Ethanol also can lower ICSS reward thresholds (28–31). Using a discrete-trial current-intensity threshold procedure, Kornetsky and coworkers (28, 29) showed that ICSS reward thresholds were lowered in animals that were allowed to self-administer ethanol orally, but not in animals receiving intraperitoneal injections of various doses of ethanol. Lewis and coworkers (30, 31), using a continuous reinforcement/fixed ratio schedule, demonstrated that intraperitoneal injections could also lower ICSS reward thresholds, but only when threshold determinations were made on the ascending limb of the blood alcohol level (BAL) curve (i.e., when the BAL was rising). While the acute effects of ethanol on ICSS reward

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: ICSS, intracranial self-stimulation; BAL, blood alcohol level; CNS, central nervous system.

*To whom reprint requests should be addressed.

thresholds had been well characterized in these earlier studies, it has been unknown whether ethanol withdrawal is characterized by an opposing response of elevations in ICSS thresholds. Therefore, the current study was designed to test the hypothesis that withdrawal from chronic exposure to ethanol also produces an elevation in reward thresholds.†

MATERIALS AND METHODS

Animals. Male Wistar rats (Charles River Breeding Laboratories) weighing 280–300 g at the start of the experiment were used as experimental subjects (n = 15). All rats were group housed (2–3 per cage) in temperature- and humidity-controlled rooms with a 12 hr light/12 hr dark cycle (lights on at 06:00). All rats had free access to food and water at all times. In addition, the rats received a dietary supplement in the form of Sustacal (Mead Johnson) mixed with an equal part of water on a daily basis while in the vapor chambers; this palatable liquid dietary supplement helped maintain body weights during ethanol vapor exposure.

ICSS. The surgery, procedure, and apparatus have been described in detail (25, 32). For surgery, 15 rats were anesthetized with halothane and a stainless-steel bipolar electrode (Plastics One, Roanoke, VA) was implanted in the lateral hypothalamus unilaterally (coordinates: anterior–posterior, 0.5 mm from bregma; lateral, 1.7 mm; 8.3 mm ventral from dura, incisor bar 5.0 mm above interaural line). To counterbalance any possible brain asymmetries, half the rats received implants on the right side of the brain, the other half on the left side.

The procedure for measuring reward thresholds was a modification of the Kornetsky and Esposito (22) discrete-trial current-threshold procedure. Stimulation was delivered by constant-current stimulators using 60-Hz sinusoidal waves, with a train duration set at 250 msec. To start each trial, a rat received a noncontingent electrical stimulus. A positive response was recorded if the rat rotated a wheel manipulandum at least one-quarter turn within 7.5 sec of the noncontingent electrical stimulus; each positive response produced a contingent stimulus identical in all parameters to the noncontingent stimulus. After each positive response, there was an intertrial interval (ITI) averaging 10 sec (7.5–12.5 sec). If no response occurred within 7.5 sec of the noncontingent stimulus, the ITI followed and that trial ended. Any responding during the ITI resulted in a 10-sec delay before the start of the next trial.

Stimulus intensities varied according to the method of limits and were presented in alternating ascending and descending series (two of each) with a step size of 5 μA; a given stimulus intensity was presented three times within each series. The threshold for each series was defined as the midpoint between the current intensity level at which at least two positive responses occurred and the level at which fewer than two positive responses occurred; the mean of the four series thresholds served as the estimated threshold for a given session. The duration of each ICSS session was ≈30 min. Stable baseline thresholds (defined as ±10% of mean on three consecutive days) were established for all rats prior to placement into the vapor chambers (see below).

Ratings of Overt Withdrawal Signs. The following well-characterized signs of ethanol withdrawal (33–37) were rated immediately after each ICSS threshold determination in ethanol-withdrawing rats: (*i*) hyperirritability upon touch, (*ii*) presence of the ventromedial distal flexion response, (*iii*) tail stiffness/rigidity, and (*iv*) abnormal posture or gait. Each sign was rated during a 3- to 5-min observation period on a scale of 0–2, with 0 indicating not detectable, 1 indicating mild to moderate incidence, and 2 indicating pronounced or severe incidence. The scores for all signs were then cumulated to yield an overall rating of withdrawal severity ranging from 0 to 8.

Vapor Inhalation. The vapor inhalation chamber apparatus and procedure were similar to those described in detail previously (38). In brief, ethanol vapor was created by dripping 95% (vol/vol) ethanol into 2-liter Erlenmyer flasks warmed to 50°C on a warming tray. Air was blown over the bottom of the flasks at a rate of 11 liters/min. Ethanol vapor concentrations were adjusted by varying the rate at which ethanol was pumped into the flask, according to the procedure described by Rogers *et al.* (38).

Ethanol vapor was introduced into sealed Plexiglas chambers through a stainless-steel manifold; each chamber received a separate supply of ethanol and air. Air and ethanol vapor could exit each chamber through holes in the chamber bottom; from there, the vapor was ported through a dedicated exhaust line to the outside air. Each chamber could hold two standard rat cages, for a total of four rats per chamber (two rats per cage).

Dependence Induction. Rats were placed into the vapor chambers and exposed initially to a moderate concentration of ethanol vapor (22 mg/liter) to allow acclimatization to breathing ethanol-laden air and a gradual rise in BAL. Tail blood was sampled every 2–3 days to determine the BALs of the subjects, and chamber vapor concentrations were adjusted up or down to maintain average BALs according to the following schedule: day 3, 50–80 mg/dl; day 5, 100–120 mg/dl; day 7, 150–180 mg/dl; day 10, 180–220 mg/dl. From day 10 onward, average BALs were maintained at 180–220 mg/dl for at least 7 days prior to onset of withdrawal.

BAL Determination. Blood samples (0.5 ml) were collected into heparinized Eppendorf tubes and subjected to microcentrifugation to separate plasma. BALs were then determined in plasma samples by a standard NAD^+–alcohol dehydrogenase (ADH) assay kit (Sigma).

Experimental Design. A crossover within-subjects design was used, such that each subject received each treatment (ethanol and control). After establishment of stable baseline ICSS thresholds (initial baseline; see Fig. 1), rats were divided into two groups for the first experimental phase. Group 1 was made dependent on ethanol through vapor inhalation as described above, and group 2 was placed into identical Plexiglas chambers but was not exposed to ethanol vapor. After 17–20 days in the vapor chambers, the rats were removed from the chambers and ICSS thresholds were determined 2, 4, 6, 8, 12, 24, 48, 72, 96, and 120 hr later. Ethanol-dependent and withdrawing rats were rated for overt signs of withdrawal immediately after each ICSS threshold determination for the first 72 hr post-ethanol. At the conclusion of this testing sequence, baseline thresholds were again assessed for 3 days (intermediate baseline; see Fig. 1) to ensure that the thresholds had returned to initial levels prior to onset of the next experimental phase. During the following 5 days the rats remained in the colony room and were not tested.

In the second experimental phase, the vapor-chamber experience of the rats was reversed as part of the crossover design, such that group 1 was now placed into vapor chambers without ethanol exposure and group 2 was made dependent through ethanol vapor exposure. In this fashion, each rat could serve as its own control. After 17–20 days in the chambers, the withdrawal and testing procedure detailed above was repeated. At the conclusion of this experimental phase, a final baseline threshold determination was made on three consecutive days (final baseline; see Fig. 1).

Data Analysis. Comparisons of initial, intermediate, and final baseline thresholds were made separately for group 1 and group 2 (see *Experimental Design*) by using a single-factor repeated-measure analysis of variance (ANOVA). Statistical analysis of the effects of ethanol withdrawal on ICSS thresh-

†A preliminary report of these data was presented at the meeting of the Society for Neuroscience, November 12–18, 1994, Miami.

olds was conducted with data from both groups combined, using two-factor ANOVA with both vapor-chamber condition (ethanol or control) and time as repeated measures. Differences among individual means were analyzed with planned comparisons of simple main effects of vapor-chamber condition. For ratings of overt signs of withdrawal, the overall rating scores (see above) were analyzed with the nonparametric Friedman ANOVA by ranks. In addition, through simple linear regression, BAL at withdrawal, defined as the BAL determined at the time of removal from the ethanol vapor chamber (i.e., 0 hr post-ethanol), was correlated with the ICSS threshold measures and the ratings of overt signs determined at various times during withdrawal (2–72 hr post-ethanol). In all cases the level of statistical significance was set at $P < 0.05$.

RESULTS

Two of the rats were excluded from the final analyses, one because of very low BAL (40 mg/dl) at the time of withdrawal, the second because of failure to respond on the wheel manipulandum for the first 24 hr of withdrawal from ethanol. The data for the remaining 13 rats were included in all statistical analyses. The average BAL for these animals over the final 10 days of ethanol exposure was 197.29 ± 12.85 mg/dl, and the average BAL at the time of withdrawal was 226.31 ± 25.02 mg/dl.

As shown in Fig. 1, baseline thresholds taken prior to the first experimental phase, between the first and second experimental phase, and after the second experimental phase did not differ from one another in either experimental group [group 1, $F(2, 10) < 1.0$, not significant; group 2, $F(2, 12) = 2.14$, not significant]. This outcome is particularly important for group 1, which was exposed to ethanol vapor first, indicating that the data obtained from these rats during the subsequent control phase of the experiment were unaffected by prior experience with ethanol. Thus, the data for groups 1 and 2 were combined for all further statistical analyses.

As shown in Fig. 2, ethanol withdrawal produced a time-dependent increase in ICSS thresholds, with maximum increases for most rats occurring at 6–8 hr post-ethanol. A two-factor within-subjects ANOVA revealed a significant main effect of vapor-chamber condition [$F(1, 12) = 14.19, P < 0.005$], a significant main effect of time [$F(9, 108) = 4.99, P < 0.0001$], and a significant condition × time interaction [$F(9, 108) = 2.34, P < 0.02$]. Subsequent planned comparisons involving simple main effects of vapor-chamber condition revealed that thresholds were significantly elevated in comparison to control values at all time points from 2 to 48 hr post-ethanol. Thresholds returned to baseline levels at 72 hr post-ethanol.

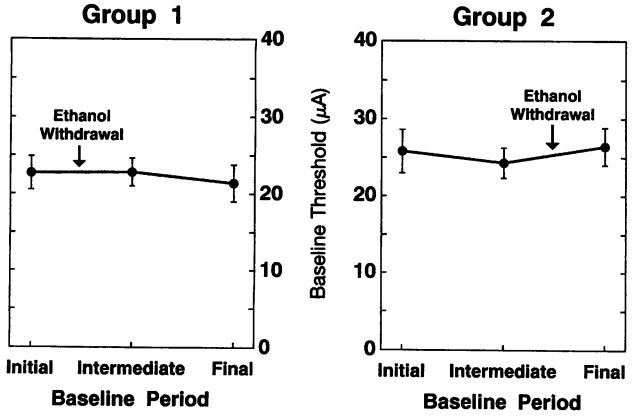

FIG. 1. Baseline ICSS thresholds at various stages of the experimental design. Baseline thresholds were defined as the average threshold on three consecutive days and were determined at the following times: initial baseline, prior to any ethanol vapor-chamber experience; intermediate baseline, between the first and second vapor-chamber experience (see *Materials and Methods*); and final baseline, at the conclusion of all experimental manipulations. There were no statistically significant changes in baseline threshold in either group 1 or group 2, which varied according to whether they were exposed to ethanol vapor first or second.

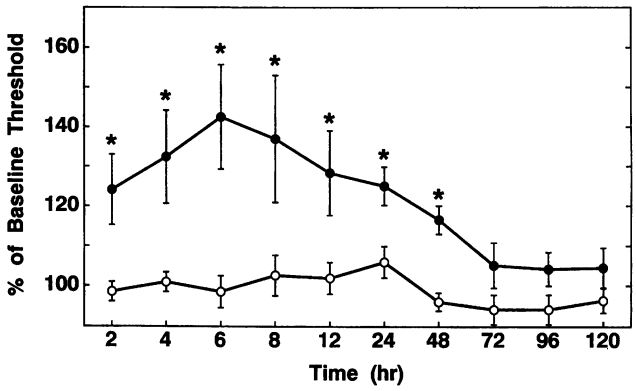

FIG. 2. Time-dependent elevation of ICSS current thresholds during ethanol withdrawal. Data are expressed as mean (± SEM) percent of baseline threshold (see Fig. 1 for mean baseline threshold values). Thresholds were significantly elevated above control levels at 2–48 hr post-ethanol (*, $P < 0.05$). ○, Control condition; ●, ethanol withdrawal condition.

The overt signs of withdrawal showed a similar time course (Table 1). Each individual sign of withdrawal was most prominent from 6 to 12 hr post-ethanol, and the overall withdrawal severity rating peaked from 6 to 8 hr post-ethanol. A Friedman ANOVA by ranks revealed a significant time-dependent effect of ethanol withdrawal on the overall rating of withdrawal severity ($\chi r^2 = 29.51, P < 0.0001$).

Correlations of BALs at withdrawal (0 hr post-ethanol) with the ICSS threshold measure and the overall rating of withdrawal severity revealed dose-dependent effects, in the sense that greater BALs were associated with higher ICSS thresholds and higher overall withdrawal rating scores during withdrawal (Table 2). The correlations of BALs at withdrawal and ratings of overt signs were significant at all time points. As would be expected, the correlation was negative at 2 hr post-ethanol, because the animals with the highest initial BALs still had significant blood levels of ethanol. The correlations of BALs at withdrawal and ICSS thresholds were significant at the peak times of withdrawal (4–12 hr).

DISCUSSION

The results demonstrate that withdrawal from chronic ethanol exposure is accompanied by elevations of ICSS reward thresholds. This effect was time dependent, peaking at 6–8 hr after abrupt withdrawal from ethanol and disappearing by 72 hr post-ethanol (Fig. 2). Furthermore, during the period of maximal threshold elevations (4–12 hr post-ethanol), this effect was positively correlated with BAL at the time of withdrawal. The time course of withdrawal also corresponded well to that obtained from more traditional measures of ethanol withdrawal (Table 1). Importantly, the effect of ethanol withdrawal on ICSS reward thresholds was opposite in direction to the effects of acute ethanol administration (28–31), providing support for an opponent-process neuroadaptation during the induction of dependence on ethanol.

It has been argued (16, 39) that the only common principle that emerges in the search for a general theory of addiction from the study of diverse classes of abused drugs, such as sedative–hypnotics, opiates, and psychostimulants, is that all appear to stimulate brain reward circuitry which connects the

Table 1. Summary of ratings of overt signs of withdrawal

	Time post-ethanol, hr							
	2	4	6	8	12	24	48	72
Overall withdrawal rating								
Mean	2.08	3.23	4.15	4.00	3.69	3.38	2.31	2.00
Range	0–4	1–6	1–8	1–8	1–7	1–7	1–6	0–5
Percent of subjects with individual signs								
Hyperirritability								
Intensity 1	38.5	53.8	46.2	46.2	38.5	46.5	53.8	53.8
Intensity 2	38.5	38.5	53.8	53.8	61.5	46.5	30.8	23.1
VMD response*								
Intensity 1	30.8	30.8	38.5	15.4	15.4	23.1	23.1	23.1
Intensity 2	0	15.4	30.8	30.8	30.8	23.1	7.7	15.4
Tail stiffness								
Intensity 1	61.5	38.5	38.5	46.2	46.2	30.8	23.1	7.7
Intensity 2	0	30.8	46.2	30.8	23.1	38.5	15.4	7.7
Abnormal gait/posture								
Intensity 1	7.7	15.4	15.4	30.8	38.5	23.1	15.4	7.7
Intensity 2	0	7.7	7.7	15.4	0	0	0	0

Each of four individual overt signs of withdrawal were rated on a scale of 0 to 2, with 0 indicating not detectable, 1 indicating moderate severity, and 2 indicating pronounced severity. The ratings for each individual sign were then cumulated to yield an overall score for withdrawal severity (range of possible values, 0–8). The ratings were made on rats undergoing ethanol withdrawal (n = 13), immediately after completion of an ICSS threshold determination session.
*Ventromedial distal limb flexion response.

ventral midbrain to the basal forebrain. According to this view, theories which attempt to incorporate dependence mechanisms into a unitary theory of addiction fall short because the classical withdrawal syndromes for sedative–hypnotics, opiates, and psychostimulants are so radically different (40). However, Edwards (8), in reviewing the literature on alcohol withdrawal, has suggested that when the ability of alcohol withdrawal to reinforce drinking behavior is considered, it is "unsatisfactory to approach the question only in terms of a global withdrawal severity. It may be necessary to consider the reinforcing potential of separate elements within the total syndrome." A similar argument can be made for other classes of abused drugs as well (5, 9, 11, 12, 18, 27, 41). Since the level of dependence induced in the current study resulted in alterations in both brain reward systems and physical signs of withdrawal, it is not possible to directly dissociate these two factors when only these data on ethanol withdrawal are considered. However, previous studies have shown that ICSS threshold elevations can be seen during withdrawal from cocaine or morphine under conditions where few (if any) physical signs of withdrawal are observed (23, 25, 27).

Taken together, the results with opiates, psychostimulants, and ethanol support the notion that alterations in brain reward systems, expressed as affective motivational signs of withdrawal, may be common to dependence on multiple classes of abused drugs but that physical signs of withdrawal are not necessarily a concomitant of such affective neuroadaptations (1, 2, 5–13, 18, 23–27).

Table 2. Correlations of withdrawal signs with BAL at withdrawal

Time post-ethanol, hr	r value	
	ICSS threshold elevation	Ratings of overt withdrawal signs
2	0.14	−0.55*
4	0.60*	0.58*
6	0.62*	0.58*
8	0.50	0.72*
12	0.55*	0.70*
24	0.09	0.77*
48	0.46	0.64*
72	0.17	0.61*

The r values are Pearson product-moment correlation coefficients. A single blood alcohol measure for each rat, taken at the time of removal from the ethanol vapor chambers (0 hr post-ethanol), was correlated with ICSS thresholds and overt withdrawal ratings determined at the indicated times during withdrawal (2–72 hr post-ethanol).
*$P < 0.05$.

With regard to a neural basis for the affective symptoms of withdrawal from drugs of abuse, Koob and Bloom (17) have argued that, as dependence develops, neuroadaptations occur within the same brain circuits that mediate the reinforcing or rewarding effects of these drugs following acute administration, leading to the expression of negative affective signs of withdrawal upon drug abstinence. There is some neuropharmacological evidence to suggest that withdrawal from ethanol, opiates, and psychostimulants may lead to similar alterations in reward circuitry. For example, cocaine withdrawal is accompanied by decreases in dopamine release in the nucleus accumbens, and the time course of this alteration in dopamine transmission correlates with the time course of ICSS threshold elevations produced by cocaine withdrawal (25, 42). Withdrawal from opiates and ethanol also results in decreases in dopamine transmission in the nucleus accumbens (43–47). Whether such changes in dopamine transmission common to withdrawal from psychostimulants, ethanol, and opiates are sufficient to account for the negative affective consequences of withdrawal is not known; it is possible that other neurochemical systems within the brain reward circuitry may also contribute to these effects. The identification of a reliable reward deficit (i.e., ICSS threshold elevations) during withdrawal from multiple drugs of abuse should prove valuable in the elucidation of the neural substrates that may be common to dependence on and withdrawal from multiple classes of abused drugs.

In conclusion, the effect of ethanol withdrawal on ICSS thresholds in rats reported herein mimics the effects of psychostimulant withdrawal (24, 25) and opiate withdrawal (26, 27). Furthermore, the elevations in reward thresholds that accompany withdrawal from these drugs are in all cases opposite in nature to the acute drug effects, providing support for neuroadaptive changes within brain reward systems during establishment of dependence. In the search for general principles of drug addiction, these data provide evidence that negative affective signs of withdrawal may be a common feature of addiction in addition to the established common

feature of the rewarding properties of abused drugs. Thus, the neural circuits that contribute to the acute rewarding effects of drugs may also be important for the motivational consequences of drug withdrawal (17). Identification of the mechanisms which contribute to such neuroadaptations within the reward circuitry may provide a key for understanding not only drug addiction but also how dysfunction of the reward system may contribute to other psychopathologies of mood and affect.

This work was supported by National Institute on Alcohol Abuse and Alcoholism Grant AA08459 (G.F.K.), Center Grant AA06420, Training Grant AA07456 (Floyd E. Bloom), and National Institute on Drug Abuse Scientist Development Award DA00213 (A.M.). This is publication no. 9121-NP from The Scripps Research Institute.

1. Goodwin, D. W. (1992) in *Substance Abuse: A Comprehensive Textbook*, eds. Lowinson, J. H., Ruiz, P., Millman, R. B. & Langrod, J. G. (Williams & Wilkins, Baltimore), pp. 144–151.
2. Jaffe, J. H. (1990) in *The Pharmacological Basis of Therapeutics*, eds. Goodman-Gilman, A., Rall, T. W., Nies, A. S. & Taylor, P. (Pergamon, Elmsford, NY), pp. 522–573.
3. Mendelson, J. H. & Mello, N. K. (1979) *N. Engl. J. Med.* **301,** 912–921.
4. Jaffe, J. H. (1992) in *Substance Abuse: A Comprehensive Textbook*, eds. Lowinson, J. H., Ruiz, P., Millman, R. B. & Langrod, J. G. (Williams & Wilkins, Baltimore), pp. 186–194.
5. Weddington, W. W., Brown, B. S., Haertzen, C. A., Cone, E. J. & Dax, E. M. (1990) *Arch. Gen. Psychiatry* **47,** 861–867.
6. American Psychiatric Association (1994) *DSM IV* (Am. Psychiatric Assoc. Press, Washington, DC).
7. Bokstrom, K. & Balldin, J. (1992) *Alcohol. Clin. Exp. Res.* **16,** 241–249.
8. Edwards, G. (1990) *Br. J. Addict.* **85,** 447–461.
9. Gawin, F. H. & Kleber, H. D. (1986) *Arch. Gen. Psychiatry* **43,** 107–113.
10. Haertzen, C. A. & Hooks, N. T. (1969) *J. Nerv. Ment. Dis.* **148,** 606–614.
11. Henningfield, J. E., Johnson, R. E. & Jasinksi, D. R. (1987) in *Methods of Assessing the Reinforcing Properties of Abused Drugs*, ed. Bozarth, M. A. (Springer, New York), pp. 573–590.
12. Jasinski, D. R., Johnson, R. E. & Kocher, T. R. (1985) *Arch. Gen. Psychiatry* **42,** 1063–1066.
13. Emmett-Oglesby, M. W., Mathis, D. A., Moon, R. T. Y. & Lal, H. (1990) *Psychopharmacology* **101,** 292–309.
14. Koob, G. F. (1992) *Trends Pharmacol. Sci.* **13,** 177–184.
15. Samson, H. H. & Harris, R. A. (1992) *Trends Pharmacol. Sci.* **13,** 206–211.
16. Wise, R. A. & Bozarth, M. A. (1987) *Psychol. Rev.* **94,** 469–492.
17. Koob, G. F. & Bloom, F. E. (1988) *Science* **242,** 715–723.
18. Koob, G. F., Markou, A., Weiss, F. & Schulteis, G. (1993) *Semin. Neurosci.* **5,** 351–358.
19. Solomon, R. L. & Corbit, J. D. (1974) *Psychol. Rev.* **81,** 119–145.
20. Esposito, R. & Kornetsky, C. (1977) *Science* **195,** 189–191.
21. Esposito, R., Motola, A. H. & Kornetsky, C. (1978) *Pharmacol. Biochem. Behav.* **8,** 437–439.
22. Kornetsky, C. & Esposito, R. (1979) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **38,** 2473–2476.
23. Markou, A. & Koob, G. F. (1992) *Physiol. Behav.* **51,** 111–119.
24. Leith, N. J. & Barrett, R. J. (1976) *Psychopharmacology* **46,** 19–25.
25. Markou, A. & Koob, G. F. (1991) *Neuropsychopharmacology* **4,** 17–26.
26. Schaefer, G. J. & Michael, R. P. (1986) *Pharmacol. Biochem. Behav.* **25,** 1263–1269.
27. Schulteis, G., Markou, A., Gold, L. H., Stinus, L. & Koob, G. F. (1994) *J. Pharmacol. Exp. Ther.* **271,** 1391–1398.
28. Bain, G. T. & Kornetsky, C. (1989) *Alcohol* **6,** 499–503.
29. Moolten, M. & Kornetsky, C. (1990) *Alcohol* **7,** 221–225.
30. Lewis, M. J. (1991) in *Neuropharmacology of Ethanol: New Approaches*, eds. Meyer, R. E., Koob, G. F., Lewis, M. J. & Paul, S. M. (Birkhauser, Boston), pp. 163–178.
31. Lewis, M. J. & June, H. L. (1990) *Alcohol* **7,** 213–219.
32. Markou, A. & Koob, G. F. (1993) in *Behavioural Neuroscience: A Practical Approach*, ed. Sahgal, A. (IRL, Oxford), Vol. 2, pp. 93–115.
33. Goldstein, D. B. (1978) in *Research Advances in Alcohol and Drug Problems*, eds. Israel Y., Glaser, F. B., Kalant, H., Popham, R. E., Schmidt, W. & Smart, R. G. (Plenum, New York), Vol. 4, pp. 77–109.
34. Goldstein, D. B. & Pal, N. (1971) *Science* **172,** 288–290.
35. Hunter, B. E., Boast, C. A., Walker, D. W. & Zornetzer, S. F. (1973) *Pharmacol. Biochem. Behav.* **1,** 719–725.
36. Majchrowicz, E. (1975) *Psychopharmacologia* **43,** 245–254.
37. Majchrowicz, E. & Hunt, W. A. (1976) *Psychopharmacology* **50,** 107–112.
38. Rogers, J., Wiener, S. G. & Bloom, F. E. (1979) *Behav. Neural Biol.* **27,** 466–486.
39. Wise, R. A. (1988) *J. Abnorm. Psychol.* **97,** 118–132.
40. Stolerman, I. P. & Goldberg, S. R. (1986) in *Behavioral Analysis of Drug Dependence*, eds. Goldberg, S. R. & Stolerman, I. P. (Academic, Orlando, FL), pp. 1–7.
41. Brady, J. V. (1990) in *NIDA Res. Monogr.* **95,** 131–134.
42. Weiss, F., Markou, A., Lorang, M. T. & Koob, G. F. (1992) *Brain Res.* **593,** 314–318.
43. Smith, A., Parsons, L. H., Merlo-Pich, E. M., Hyytia, P., Schulteis, G., Lorang, M. T., Yackey, M., Koob, G. F. & Weiss, F. (1994) *Soc. Neurosci. Abstr.* **20,** 1614.
44. Acquas, E. & Di Chiara, G. (1992) *J. Neurochem.* **58,** 1620–1625.
45. Acquas, E., Carboni, E. & Di Chiara, G. (1991) *Eur. J. Pharmacol.* **193,** 133–134.
46. Pothos, E., Rada, P., Mark, G. P. & Hoebel, B. G. (1991) *Brain Res.* **566,** 348–350.
47. Rossetti, Z. L., Melis, F., Carboni, S., Diana, M. & Gessa, G. L. (1992) *Alcohol. Clin. Exp. Res.* **16,** 529–532.