

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/13521237>

# Transition from Moderate to Excessive Drug Intake: Change in Hedonic Set Point

Article in *Science* · November 1998

Impact Factor: 33.61 · DOI: 10.1126/science.282.5387.298 · Source: PubMed

---

CITATIONS

618

---

READS

131

2 authors, including:



Serge H Ahmed

French National Centre for Scientific Research

89 PUBLICATIONS 4,010 CITATIONS

SEE PROFILE

sion (compare SVβgal and SVH2βgal) (Fig. 3E), consistent with the in vitro translation data of Fig. 3D. Expression of the luciferase reporter was also not inhibited by H33342 (19). However, H33342 reduced β-galactosidase activity from SVH2βGal by greater than 90% in a dose-dependent fashion (Fig. 3E).

We have described how a small molecule and its RNA aptamer can be used to design a translation switch for controlling gene expression in living cells. The results also establish the possibility of using small molecules to regulate expression of endogenous genes.

References and Notes

1. C. Tuerk and L. Gold, *Science* **249**, 505 (1990); A. D. Ellington and J. W. Szostak, *Nature* **346**, 818 (1990).
2. K. W. Uphoff, S. D. Bell, A. D. Ellington, *Curr. Opin. Struct. Biol.* **6**, 281 (1996); M. G. Wallis and R. Schroeder, *Prog. Biophys. Mol. Biol.* **67**, 141 (1997).
3. M. Gossen and H. Bujard, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 5547 (1992); D. No, T. P. Yao, R. M. Evans, *ibid.* **93**, 3346 (1996); J. M. Gottesfeld *et al.*, *Nature* **387**, 202 (1997); C. Liu *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 940 (1996).
4. D. M. Spencer, T. J. Wandless, S. L. Schreiber, G. R. Crabtree, *Science* **262**, 1019 (1993).
5. The initial 70-nucleotide RNA pool, containing 31 random nucleotides, was constructed as described [R. Singh, J. Valcarcel, M. R. Green, *ibid.* **268**, 1173 (1995)]. Tobramycin and kanamycin A were covalently linked to cyanogen bromide-activated Sepharose 4B. The RNA pool was dissolved in selection buffer [50 mM tris-HCl (pH 8.3), 250 mM KCl, 2 mM MgCl<sub>2</sub>], loaded onto a preselection column (0.25 ml of glycine-Sepharose), eluted with two column volumes of selection buffer, and immediately loaded onto a 0.5-ml aminoglycoside-Sepharose column. Columns were washed with 10 column volumes of selection buffer (rounds 1 to 5), 10 column volumes of buffer with 5 mM competitor aminoglycoside (rounds 6 to 9), or 10 column volumes of buffer with 10 mM competitor (rounds 10 to 14). The competitor aminoglycoside for tobramycin aptamer selection was kanamycin A and vice versa. Bound RNA was eluted with the cognate aminoglycoside (5 mM) and amplified by reverse transcriptase-polymerase chain reaction (RT-PCR) using flanking primers. The PCR products were transcribed into RNA with T7 RNA polymerase and purified by polyacrylamide gel electrophoresis. Pools were subcloned into the plasmid pBlue-script (Stratagene) and sequenced after rounds 10, 12, and 14.
6. One or three copies of the kanamycin A (kan) or the tobramycin (tob) aptamer were cloned into the Nde I site (one copy) or Nde I and Bsa I sites (three copies) of T7 RNA polymerase-driven expression vector pRSETA (Invitrogen) and transformed into a bacterial strain containing an isopropyl β-D-thiogalactopyranoside (IPTG)-inducible T7 RNA polymerase. Bacterial strains were grown in liquid culture overnight, induced with 0.1 mM IPTG for 1 hour, and then diluted into medium containing antibiotic and 0.1 mM IPTG.
7. The partial resistance of bl-kan1 to tobramycin is consistent with the fact that the kan aptamer binds both drugs, whereas the tob aptamer is more selective and binds only tobramycin.
8. None of the strains exhibited increased resistance to the unrelated antibiotics tetracycline and gentamicin.
9. M. Kozak, *Annu. Rev. Cell Biol.* **8**, 197 (1992); A. B. Sachs, P. Sarnow, M. W. Hentze, *Cell* **89**, 831 (1997).
10. R. Stripecke, C. C. Oliveira, J. E. G. McCarthy, M. W. Hentze, *Mol. Cell. Biol.* **14**, 5898 (1994); E. Paraskeva, A. Atzberger, M. W. Hentze, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 951 (1998).
11. In vitro transcription reaction mixtures contained 5 μg of pRSETA (or RSET derivative); 0.5 mM μG(5')ppp(5')G; 0.5 mM adenosine triphosphate, cytidine triphosphate, and uridine triphosphate (UTP); 0.05 mM guanosine triphosphate; 10 mM di-thiothreitol; and 40 units of T7 RNA polymerase in 50

- μl of a solution of 40 mM tris-HCl (pH 7.5), 6 mM MgCl<sub>2</sub>, 2 mM spermidine, 10 mM NaCl. After incubation for 1 hour at 37°C, RNA was purified by extraction with phenol and chloroform, precipitated with ethanol, and resuspended in 30 μl of H<sub>2</sub>O. Translation reactions were carried out in 10 μl containing 5 μl of wheat germ extract, 0.8 μl of 1 mM amino acid mixture (without methionine), 2 μl of RNA transcript as described above, [<sup>35</sup>S]methionine (0.5 μl; 1200 Ci/mmol), and 0 to 80 μM drug. Reaction mixtures were incubated at 25°C for 15 min and terminated by addition of 2× sample loading buffer. Translation products were separated by electrophoresis on an 18% polyacrylamide gel, visualized by autoradiography, and quantitated by densitometry.
12. Translation repression was more efficient with multiple aptamers than with a single aptamer. Repression was also more efficient when the aptamers were positioned near the 5' end of the mRNA.
13. A. G. Gilman, T. W. Rall, A. S. Nies, P. Taylor, Eds., *Goodman and Gilman's The Pharmacological Basis of Therapeutics* (Pergamon, Elmsford, NY, 1990).
14. K. H. Elstein and R. M. Zucker, *Exp. Cell Res.* **211**, 322 (1994); M. V. Filatov and E. Y. Varfolomeeva, *Mutat. Res.* **327**, 209 (1995).
15. Isolation of H33258 aptamers was carried out by covalently linking H33258 to epoxy-activated Sepharose 6B. The ligand solution was mixed at 37°C for 16 hours. The resin was then washed and excess active groups were blocked with 1 M ethanolamine (pH 10). Preselection columns were prepared with ethanolamine alone. H33258 selection buffer contained 50 mM tris-HCl (pH 7.3), 200 mM KCl, 2 mM MgCl<sub>2</sub>. In selection

- rounds 1 to 6, columns were washed with 20 column volumes of selection buffer and eluted with 2 column volumes of 10 mM H33258. In selection rounds 7 to 10, columns were washed with 20 column volumes of buffer and 20 column volumes of 10 mM benzimidazolepropionic acid (in selection buffer) before elution.
16. H10 and H19 bound H33258 and the closely related H33342 comparably.
17. CHO cells (80% confluent) were pretreated with 0, 5, or 10 mM H33342 and then cotransfected (Lipofectamine, Gibco-BRL) with 1 μg of pSVβgal or pH2βgal and 1 μg of the luciferase reporter gene pGL3 (Promega). Two hours after transfection H33342 was reapplied to the cells. Twenty-four hours after transfection cells were harvested and cell extracts were prepared. Cell extracts were normalized for total protein (Bradford assay). β-Galactosidase and luciferase activities in the extracts were determined relative to standard curves generated with the purified β-galactosidase and luciferase enzymes (Promega).
18. In these experiments, H33342 was used instead of H33258 because it is about 10 times more cell-permeable (14).
19. The parental expression vector SVβGal was also not inhibited by 5 or 10 μM H33342.
20. We are grateful to R. Singh for advice and for providing the randomized RNA pool and to M. Zapp for helpful discussions. This work was supported by an NIH grant to M.R.G. M.R.G. is an investigator of the Howard Hughes Medical Institute.

7 July 1998; accepted 3 September 1998

## Transition from Moderate to Excessive Drug Intake: Change in Hedonic Set Point

S. H. Ahmed\* and G. F. Koob

Differential access to cocaine self-administration produced two patterns of drug intake in rats. With 1 hour of access per session, drug intake remained low and stable. In contrast, with 6 hours of access, drug intake gradually escalated over days. After escalation, drug consumption was characterized by an increased early drug loading and an upward shift in the cocaine dose-response function, suggesting an increase in hedonic set point. After 1 month of abstinence, escalation of cocaine intake was reinstated to a higher level than before. These findings may provide an animal model for studying the development of excessive drug intake and the basis of addiction.

A critical problem in drug addiction research is to understand the differences between controlled and uncontrolled drug use, the latter being an essential feature of drug addiction (1, 2). These two patterns of drug use may be observed simultaneously in different individuals or they may represent successive stages in the same individuals. The transition from drug use to addiction often involves a gradual process of escalated drug intake, whereby an individual's consumption becomes ex-

aggerated with chronic exposure to a drug (2). Because escalation of drug use defines a common feature of drug addiction, the study of the factors that govern its development may help to explain the transition from drug use to drug addiction.

In animal models of drug self-administration, availability plays a role in determining the pattern of drug intake, as suggested by different studies with different drug access conditions (3). Numerous studies have restricted drug access to a few hours per day and produced a regular and stable pattern of consumption. In contrast, others have shown that, with continuous access to the drug, different patterns of drug intake develop, including the binge-like patterns of psychomotor stimulant use observed in both animals and humans (3, 4). Unknown,

Division of Psychopharmacology, Department of Neuropharmacology, CVN-7, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA.

\*To whom correspondence should be addressed. E-mail: aserge@sage.scripps.edu

## REPORTS

however, are the mechanisms responsible for escalation of drug intake that may set the stage for addiction. Here, we directly assessed the effect of the history of drug use on cocaine-taking behavior and have begun to characterize the factors responsible for the development of escalation of cocaine use and its reversal and relapse.

The duration of access to cocaine (250  $\mu$ g per infusion) dramatically influenced intake (5). With short access (ShA), cocaine intake remained stable over time, whereas with long access (LgA), cocaine intake gradually escalated from 71 to 110 infusions (Fig. 1A). Escalation of cocaine use appeared as early as the first hour of access to the drug (Fig. 1B), which rules out an acute within-session tolerance (6). Furthermore, increased cocaine use did not result from an increase in general activity (7). Within the first hour, the temporal course of cocaine intake was unchanged in ShA rats with repeated testing (Fig. 1C); in LgA rats with repeated testing, cocaine intake increased dramatically the first 10 min and then dropped and stabilized at a larger amount (Fig. 1D). This increased drug-loading behavior may reflect an acquired "need state" for a higher level of cocaine intoxication (1).

To establish the generality of these observations and to further determine the nature of the changes underlying increased cocaine use, escalated intake was produced in a different experimental condition (8). Again, changing the duration of drug availability induced two patterns of cocaine intake (Fig. 2, A and B). Escalation of total cocaine intake showed no sign of stabilization even after 22 sessions of self-administration (Fig. 2A). Moreover, the temporal pattern of cocaine intake in LgA rats peaked in the first 10 min and then dropped to stabilize at an amount higher than that in ShA rats. Therefore, escalated cocaine use is a robust phenomenon that is observable under different experimental conditions.

In the simple reinforcement schedule used here, the cocaine dose-response curve has a negative slope; above a certain threshold dose, an increase in the unit dose produces a proportional decrease in self-infusions (9). This phenomenon suggests that the animals regulate their intoxication around some endogenous reference or "hedonic set point" (1, 9). It is possible that escalated cocaine use results from elevation of a hedonic set point, a hypothesis consistent with the dramatic drug-loading behavior described previously (Fig. 1D). This hypothesis also predicts that the entire dose-response curve should be shifted upward after escalation. Although drug tolerance (or even sensitization) could account for some aspects of increased use, pharmacological tolerance or sensitization usually reflects a change in drug sensitivity that translates into a horizontal shift of the dose-response curve either to the right (toler-

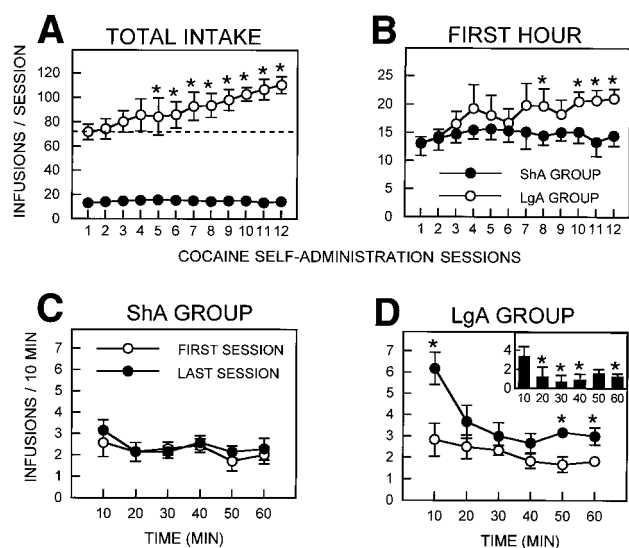
ance) or to the left (sensitization) (10, 11).

To test these predictions, we examined a wide range of cocaine doses (12). Decreasing the dose produced an increase in cocaine self-infusions (Fig. 2C), a phenomenon that is true for both ShA and LgA rats. This compensatory process ceased abruptly when the dose was too low to maintain responding (that is, when responding for this dose was similar to responding for vehicle, indicated by the broken line in Fig. 2C). Importantly, the minimum dose able to maintain cocaine-taking behavior (31.25  $\mu$ g per infusion) did not differ between groups, suggesting that drug sensitivity was unchanged (Fig. 2C). In fact, escalated cocaine use produced an upward shift without a horizontal shift in the

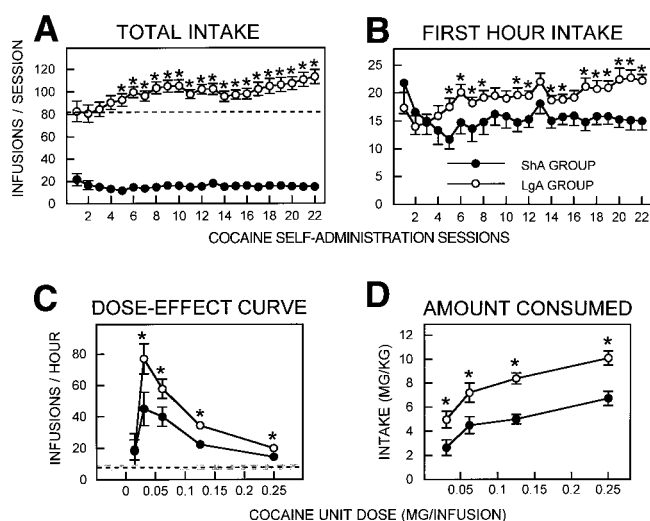
dose-response curve. At all doses tested above the threshold, LgA rats self-administered more cocaine than ShA rats (Fig. 2D). This effect did not result from an increase in general activity, because both groups responded similarly for vehicle (12). These findings suggest that escalated cocaine use results from a change in the hedonic set point for cocaine instead of from a simple change in sensitivity to the drug (1). This change induced by increasing drug availability suggests that an even longer access to the drug could lead to a further increase in set point.

To assess the persistence of escalated cocaine use, drug access was interrupted for 35 days and then restored (13). In ShA rats, abstinence had no effect on cocaine intake

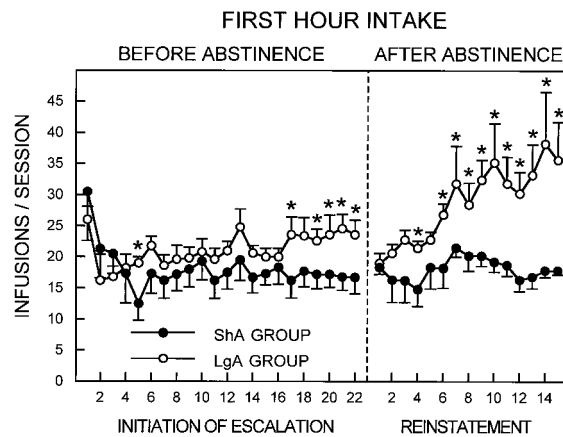
**Fig. 1.** Effect of drug availability on cocaine intake (mean  $\pm$  SEM). (A) In LgA rats ( $n = 6$ ) but not in ShA rats ( $n = 7$ ), total cocaine intake started to increase significantly from the fifth session ( $P < 0.05$ ; sessions 5 to 12 compared with session 1) and continued to increase thereafter ( $P < 0.05$ ; session 5 compared with sessions 10 to 12). (B) During the first hour, LgA rats took progressively more infusions than ShA rats ( $P < 0.05$ ; sessions 8, 10, 11, and 12). (C and D) In LgA rats (D) but not in ShA rats (C), cocaine intake measured at 10, 50, and 60 min increased ( $P < 0.05$ ; first session compared with last session). As shown in the inset in (D), this change (last minus first session) was greater the first 10 min ( $P < 0.05$ ; compared with the first 10 min). \*,  $P < 0.05$  (Student's  $t$  test after appropriate one-way and two-way analysis of variance).



**Fig. 2.** Reproduction of escalated cocaine use in another setting. (A) In LgA rats ( $n = 12$ ) but not in ShA rats ( $n = 12$ ), mean total cocaine intake ( $\pm$  SEM) started to increase significantly from session 5 ( $P < 0.05$ ; sessions 5 to 22 compared with session 1) and continued to increase thereafter ( $P < 0.05$ ; session 5 compared with sessions 8 to 10, 12, 13, and 17 to 22). (B) During the first hour, LgA rats self-administered more infusions than ShA rats during sessions 5 to 8, 11, 12, 14, 15, and 17 to 22 ( $P < 0.05$ ). (C) Mean infusion ( $\pm$  SEM) per cocaine dose tested. LgA rats took significantly more infusions than ShA rats at doses of 31.25, 62.5, 125, and 250  $\mu$ g per infusion ( $P < 0.05$ ). (D) After escalation, LgA rats took more cocaine than ShA rats regardless of the dose ( $P < 0.05$ ). \*,  $P < 0.05$  (Student's  $t$  test after appropriate one-way and two-way analysis of variance).



**Fig. 3.** Relapse to escalated cocaine use after abstinence. During the initiation phase (left), escalated cocaine use in LgA rats ( $n = 5$ ) was significant after session 19 ( $P < 0.05$ ). During reinstatement (right), escalated cocaine intake in LgA rats was fully reinstated after only six reinstatement sessions ( $P < 0.05$ ). The session-by-session fluctuation observed in ShA rats ( $n = 6$ ) during reinstatement was not significant (simple main effect). \*,  $P < 0.05$  (Student's  $t$  test after appropriate one-way and two-way analysis of variance).



(Fig. 3). In contrast, in LgA rats, cocaine intake dropped to the quantity observed in ShA rats after abstinence, indicating a complete recovery from prior increased cocaine use. However, escalated cocaine intake was reinstated by reexposing rats to the LgA regimen. In the same rats, reinstatement of escalated cocaine intake was more dramatic than when it was originally established (compare Fig. 3, left and right). This suggests that, although abstinence promotes a short-lived return to previous levels of use, a history of drug escalation facilitates the relapse. The return of cocaine use to baseline during abstinence is reminiscent of a reversal of tolerance; however, the more dramatic escalation observed during reinstatement is more consistent with an allostatic dysregulation in hedonic set point (1).

This study demonstrates the existence of two patterns of cocaine-taking behavior. Rats allowed short access to cocaine maintained a stable and low intake for many weeks, and this demonstrates that self-administering a drug repeatedly does not lead obligatorily to a process of escalated use analogous to that described in human drug addicts. This observation shows the limitations of equating drug-taking behavior per se with drug addiction and of analyzing drug addiction only in terms of the positive-reinforcing effects of a drug (1). Indeed, positive-reinforcement analyses explain how behavior is initiated and maintained by a drug but do not explain why an individual self-administers a particular amount of drug and why such an amount may or may not change over time.

In this context, the escalation in drug intake observed in rats that had longer access to cocaine (6 hours) may offer a better animal model of the transition of drug use to drug addiction. Escalated cocaine use produced an increase in drug loading and an upward shift in the cocaine dose-effect function, suggesting an increase in hedonic set point as a

potential mechanism for increased drug use. Animals, and perhaps human addicts, may take more cocaine after escalated intake not because they are simply tolerant to its rewarding effects but because they are trying to reach and then to maintain a higher state of intoxication. Certainly, tolerance, as classically defined, may contribute to this allostatic dysregulation; however, the current hypothesis is that an increase in hedonic set point would characterize the transition to the addicted state (1).

Finally, the temporal course of escalated drug use suggests that the "switch mechanism" of the transition to drug addiction may be gradual rather than abrupt. This is consistent with the slow accumulation of some drug-responsive gene products in the brain described recently during chronic exposure to cocaine (14). As shown by the dramatic reinstatement of escalated cocaine use after abstinence, part of these modifications are long lasting. Analysis of the brain changes responsible for the escalation in self-administration of cocaine observed in this animal model may help to explain the chronic, relapsing nature of drug addiction.

**References and Notes**

1. G. F. Koob, *Neuron* **16**, 893 (1996); \_\_\_\_\_ and M. Le Moal, *Science* **278**, 52 (1997); A. I. Leshner, *ibid.*, p. 45.
2. G. Edwards, *Br. J. Addict.* **81**, 171 (1986); F. Gawin, *Science* **251**, 1580 (1991); G. A. Marlatt, J. S. Baer, D. M. Donovan, D. R. Kivlahan, *Annu. Rev. Psychol.* **39**, 223 (1988); C. P. O'Brien, R. Ehrman, J. W. Ternes, in *Behavioral Analysis of Drug Dependence*, S. R. Goldberg and I. P. Stolerman, Eds. (Academic Press, Orlando, FL, 1986), pp. 329-356.
3. A. M. Young and S. Herling, in *Behavioral Analysis of Drug Dependence*, S. R. Goldberg and I. P. Stolerman, Eds. (Academic Press, Orlando, FL, 1986), pp. 9-67.
4. M. A. Bozarth and R. A. Wise, *J. Am. Med. Assoc.* **254**, 81 (1985); G. Deneau, T. Yanagita, M. H. Seevers, *Psychopharmacologia* **16**, 30 (1969); F. Gawin and E. H. Ellinwood, *N. Engl. J. Med.* **318**, 1173 (1988); C. E. Johanson, R. L. Balster, K. Bonese, *Pharmacol. Biochem. Behav.* **4**, 45 (1976).
5. The apparatus and the detailed procedures for both intravenous catheterization and self-administration of

cocaine are described in S. H. Ahmed and G. F. Koob [*Psychopharmacology* **132**, 289 (1997)]. Briefly, adult male Wistar rats (280 to 330 g) were trained 2 hours per day for 14 days on a fixed-ratio 1: time-out 20-s schedule of cocaine self-administration (250  $\mu$ g per infusion in a volume of 0.1 ml delivered in 4 s). Two noncontingent infusions of the training dose signaled the start of each session. After a predetermined acquisition criterion was reached (at least 15 self-infusions in 2 hours), subjects were divided into two balanced groups. In one group drug access was reduced to 1 hour per session (ShA group); in the other group drug access time was increased to 6 hours (LgA group). This was continued for 12 sessions performed daily, except when days off were imposed to prevent significant weight loss in LgA rats.

6. Acute tolerance is also incompatible with the stable hourly drug intake observed during the last 5 hours of the 6-hour session.
7. General activity was measured on a second, but inactive, lever. A two-way analysis of variance performed on the last three sessions, when LgA rats took consistently more cocaine than ShA rats, revealed neither a significant group effect ( $F_{1,11} = 1.23$ ) nor a significant group-by-session interaction ( $F_{2,22} = 0.98$ ).
8. Main differences with the first experiment were as follows. First, rats (320 to 460 g) had no prior history of cocaine self-administration but were pretrained to press a lever for food; see S. H. Ahmed and G. F. Koob [*Psychopharmacology* **132**, 289 (1997)]. Second, during the escalation phase, cocaine (250  $\mu$ g per infusion) was available every other day. Third, no noncontingent infusions were given except on rare occasions when a subject failed to respond during the first 10 min. Finally, some subjects were recatheterized and allowed at least 2 days of recovery (seven ShA rats; six LgA rats).
9. R. Pickens, R. A. Meisch, T. Thompson, in *Handbook of Psychopharmacology*, L. L. Iversen, S. D. Iversen, S. H. Solomon, Eds. (Plenum Press, New York, 1978), pp. 1-37; R. A. Yokel, in *Methods of Assessing the Reinforcing Properties of Abused Drugs*, M. A. Bozarth, Ed. (Springer-Verlag, New York, 1987), pp. 211-227.
10. J. Altman et al., *Psychopharmacology* **125**, 285 (1996); A. J. Goudie and M. W. Emmett-Oglesby, *Psychoactive Drugs: Tolerance and Sensitization* (Humana Press, Clifton, NJ, 1989); H. Kalant, A. E. Le Blanc, R. J. Gibbins, *Pharmacol. Rev.* **23**, 135 (1971).
11. Previous work has shown that chronic exposure to cocaine can produce tolerance to its reinforcing effects [M. W. Emmett-Oglesby and J. D. Lane, *Behav. Pharmacol.* **3**, 193 (1992); M. W. Emmett-Oglesby et al., *Drug Alcohol Depend.* **32**, 247 (1993)]. Nevertheless, such changes in a dose-effect function may also support a set-point hypothesis.
12. Cocaine doses were tested during 3-hour testing sessions every other day between regular self-administration sessions. Doses were tested once in the following order: 250, 31.2, 15.6, 62.5, 125, and 0  $\mu$ g per infusion. Because rats almost ceased to respond 2 hours after cocaine was replaced by vehicle, only the third hour was considered for analysis. Responding for vehicle was averaged regardless of the experimental group [vehicle responses (mean  $\pm$  SEM) were 7.33  $\pm$  1.1 and 6.75  $\pm$  1.36 for ShA rats and LgA rats, respectively].
13. After the dose-response study, rats were subjected to 35 days of total abstinence. After abstinence, rats with a patent catheter (six ShA rats and five LgA rats) were reexposed to the initial drug access condition.
14. G. F. Koob and E. J. Nestler, *J. Neuropsychiatr. Clin. Neurosci.* **9**, 482 (1997); E. J. Nestler and G. K. Aghajanian, *Science* **278**, 58 (1997).
15. Supported by National Institute on Drug Abuse grants DA04398 and DA08467 (G.F.K.). We thank R. Lintz and M. Beschen for their invaluable technical assistance; L. Gold, C. Heyser, N. Gracy, A. Markou, L. Parsons, J. Walker, M. Weed, and F. Weiss for their helpful comments on an early version of this manuscript; and M. Arends for correcting the remaining errors. This is publication 11573-NP from The Scripps Research Institute.

13 April 1998; accepted 7 September 1998