Pharmacology Biochemistry & Behavior, Vol. 29, pp. 157–162. © Pergamon Journals Ltd., 1988. Printed in the U.S.A. 0091-3057/87 $3.00 + .00

An Analysis of Cocaine Effects on Locomotor Activities and Heart Rate in Four Inbred Mouse Strains

JAMES A. RUTH, ELIZABETH A. ULLMAN AND ALLAN C. COLLINS

The School of Pharmacy and The Institute for Behavioral Genetics
The University of Colorado, Boulder, CO 80309-0297

Received 6 April 1987

RUTH, J. A., E. A. ULLMAN AND A. C. COLLINS. *An analysis of cocaine effects on locomotor activities and heart rate in four inbred mouse strains.* PHARMACOL BIOCHEM BEHAV **29**(1) 157–162, 1988.—The effects of cocaine on Y-maze activity and heart rate have been examined in four inbred strains of mouse (BALB, C57BL, C3H and DBA). In addition, brain [^{3}H]-cocaine concentrations were measured at the time of maximal response to cocaine. Cocaine produced a dose-related increase in Y-maze cross activity in C3H, DBA and C57BL, with C3H mice being considerably more sensitive than DBA or C57BL. Cocaine was without effect on Y-maze cross activity in BALB mice. Cocaine produced a biphasic effect on rearing activity in C3H mice, a dose related depression in BALB mice, and was without effect on C57BL and DBA mice. At the highest dose studied (15 mg/kg), cocaine produced a small decrease in heart rate in C3H mice. Strain differences in behavior were maximal 15 minutes after a dose of 5 mg/kg, IP. At this dose and time interval, brain [^{3}H]-cocaine concentrations were not significantly different among the four strains of mice. The results suggest a genetically-determined difference in CNS sensitivity to cocaine.

Cocaine Genetics Locomotor effects Heart rate [^{3}H]-cocaine brain concentration

COCAINE use and abuse has become widespread in our society, but little is known concerning the biological factors that contribute to the use of this substance. Genetic factors are known to contribute to the use of other psychoactive substances by humans. For example, it has been clearly established that genetic factors contribute to the development of alcoholism [4, 5, 11] and it may be that the use of tobacco products is also regulated, in part, by heritable factors [8,26]. Similarly, significant variability in response to the methylxanthines (caffeine, theophylline) exist in humans [9,30] and it has been suggested that these differences, which are presumably genetically determined, explain why some people use methylxanthines and others do not. No studies of the potential influence of genotype on cocaine use by humans have been published.

One way of establishing the potential for genetic factors influencing substance use and abuse in humans is to ascertain whether variability exists in parameters such as sensitivity to an acute dose of the drug, the development of drug tolerance or drug avidity in genetically-defined animal models. Inbred strains of the mouse or rat have been used frequently for such studies. If genetic factors regulate the acute sensitivity, tolerance development or drug avidity (self-administration) in laboratory animals the possibility exists that humans may also differ in such measures because of genetically regulated factors.

Numerous studies have demonstrated that genetically-defined stocks of animals, generally mice or rats, differ in acute sensitivity to alcohol, in the development of tolerance to alcohol, in alcohol withdrawal and in alcohol self-administration (see [6, 10, 14, 15, 24, 29] for examples). Strain differences in response to other CNS depressants such as the barbiturates [1], benzodiazepines [7], general anesthetics [16], and opiates [3,27] have also been reported. Similarly, strain differences have been reported for a number of CNS stimulants including the methylxanthines [25], amphetamine [20,22], apomorphine [17], and nicotine [12, 18, 19, 21]. Strain differences in response to drugs could result from differences in pharmacokinetic and/or pharmacodynamic measures and, with only a few exceptions, such determinations have not been made. Nonetheless, the data currently available clearly indicate that genetic factors regulate the behavioral responses, both acute and chronic, that accompany psychoactive drug administration.

The studies presented here comprise an attempt to establish whether inbred mouse strains differ in their responses to an acute dose of cocaine. These studies used four inbred mouse strains that we have demonstrated differ in acute sensitivity [12, 18, 21] and in development of tolerance [19] to nicotine. Two behavioral responses, Y-maze locomotor and rearing activities, and a physiological response, heart rate, were measured. In an attempt to ascertain whether the strain differences that were detected were due to pharmacokinetic or pharmacodynamic differences, brain levels of radiolabeled cocaine were measured at the time of behavioral testing (15 min after injection).

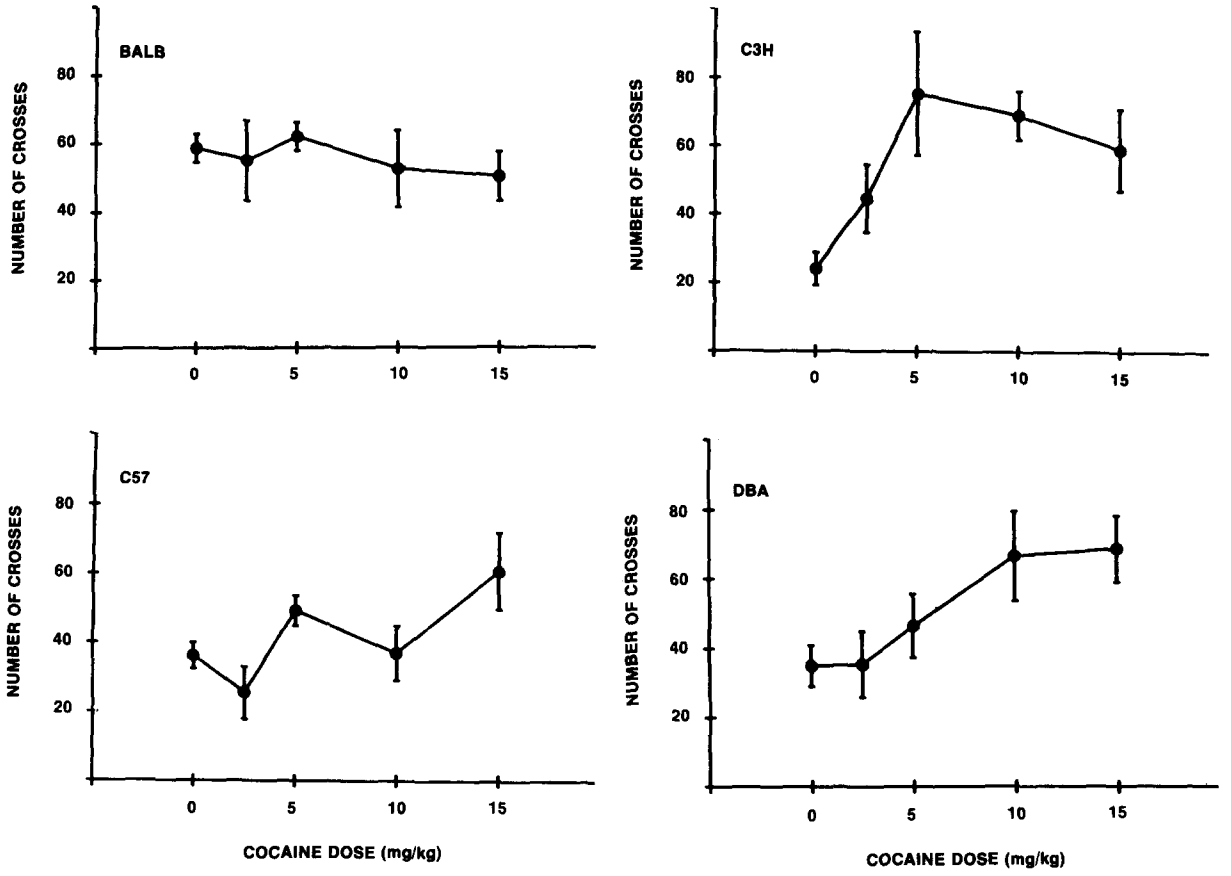

FIG. 1. Dose related effect of cocaine on Y-maze crossing activity of four inbred mouse strains. Cocaine in normal saline was injected IP. After 15 minutes the animals were placed in a Y-maze as described in the experimental section. Crosses from one of the six sections of the maze to another were counted for a period of three minutes. Values represent the mean±S.E.M. of observations on 6–8 animals.

METHOD

Animals

Male mice of four inbred strains were used in this study. C57BL/6Ibg, DBA/2Ibg and C3H/2Ibg mice were bred at the Institute For Behavioral Genetics, University of Colorado, Boulder, CO. These strains have been maintained at the Institute for at least 20 generations. Male mice of the BALB/cByJ strain, originally obtained from Jackson Laboratories, Bar Harbor, ME, were also bred at the Institute, but have been maintained there for fewer than 10 generations. All animals were weaned at 25 days of age and were housed with 1–5 male littermates. Food (Wayne Lab Blox) and water were available ad lib. Animals were 60–90 days old when tested.

Cocaine Administration

Cocaine HCl was obtained from Sigma Chemical Co., St. Louis, MO. The drug was dissolved in normal saline, and was administered by intraperitoneal (IP) injection. The injection volume was 0.01 ml/g body weight. Five doses were used: 0.0, 2.5, 5.0, 10.0 and 15.0 mg/kg. When employed, L-[benzoyl-3,4-^{3}H]cocaine (29.9 Ci/mmol, New England Nuclear Corp., Boston, MA) was used in the preparation of [^{3}H]-cocaine for determination of brain concentrations after IP administration. For these experiments, the 5 mg/kg dose was administered at a specific activity of 7,485 dpm/nmol.

Y-Maze Activity

Both locomotor (crossing) and rearing activity were measured in a symmetrical Y-maze. The maze consists of three arms which are 26 cm long, 6.1 cm wide and 10.2 cm high. Each arm was divided into two equal sections. The maze was constructed of black acrylic plastic, and underlit through a red floor using two 25 cm, 8 W fluorescent bulbs. The top of the Y-maze was constructed of red translucent acrylic plastic. Testing was begun 15 min after injection of cocaine by placing the mouse in the center of the maze. Testing was conducted for 3 min. Movements from one section to another were counted, as were rears. Preliminary experiments indicated that testing 15 min after injection assured near maximal response to injected cocaine.

Heart Rate

Heart rate was measured by placing a mouse in a restrainer to allow insertion of needle electrodes under the skin. One electrode was placed behind the left foreleg and the other in front of the right hindleg. The electrodes were connected through a preamplifier to a Narco Systems E & M

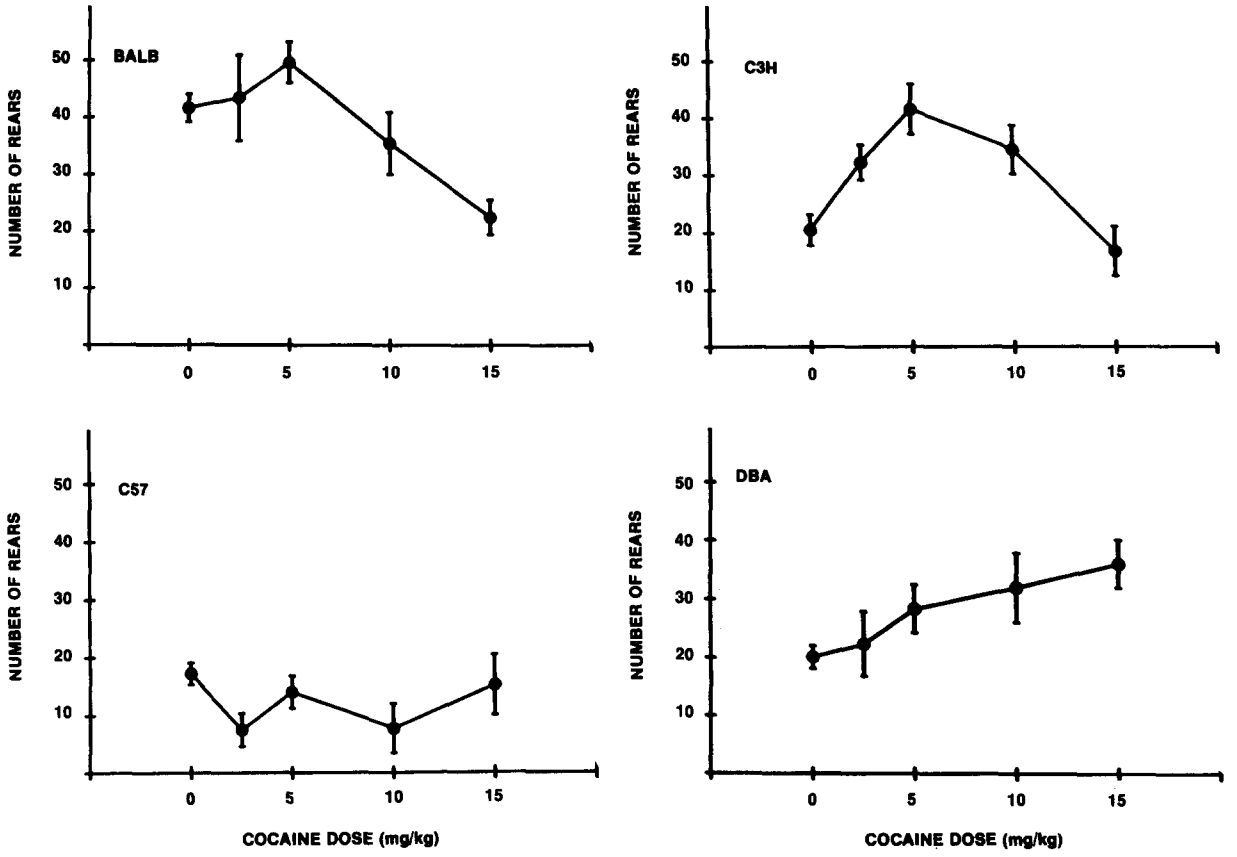

FIG. 2. The dose related effect of cocaine on Y-maze rearing activity of four inbred mouse strains. Cocaine in normal saline was injected IP. After 15 minutes, animals were placed in a Y-maze as described in the experimental section. Rearing activity was counted for a period of three minutes. Values represent the mean±S.E.M. of observations on 6–8 animals.

Physiograph. Heart rate was measured for 6 sec and the rate was estimated by counting the number of QRS complexes. Heart rate was measured 18 min after injection. These mice had already been tested in the Y-maze.

[^{3}H]-Cocaine Concentration in Brain

Animals were injected with [^{3}H]-cocaine, and were decapitated, 15 min later. The brains were quickly removed and weighed and the tissue was immediately homogenized in 3 ml of cold (4°C) 50 mM tris HCl (pH 7.4) containing 40 mM NaF to inhibit plasma esterases. The resulting suspension was centrifuged (20,000× g 20 min), and the supernatant was decanted and adjusted to pH 9.5. The supernatant was then extracted twice with 2 ml portions of ethyl acetate. In this extraction, ecgonine and benzoyl ecgonine, the major cocaine metabolites in brain, remain in the aqueous layer [2]. The organic extract was assayed by liquid scintillation counting (minimum efficiency 35%), and each sample was corrected for quenching. Extraction efficiency measured for this procedure was found to be 92.1±3.8%. Brain concentrations were calculated using the specific activity factor described above, and expressed as nmol/g of tissue.

Data Analysis

All data were analyzed using both a one-way and a two-way Analysis of Variance (ANOVA) to determine the effects of strain and dose within a test condition. Since substantial differences in basal (saline injected) Y-maze activity was found, these data were analyzed after normalizing the scores for the individual test saline activities for each of the four strains. For those analyses in which significant effects were observed, the results were subjected to Newman-Keul's *post hoc* test.

RESULTS

Figure 1 presents the effects of the varying doses of cocaine on Y-maze locomotor activity (number of crosses into the separate arms of the Y-maze) in the four strains. Both qualitative and quantitative differences among the strains were detected in this measure. C3H mice responded to cocaine injection with an overall increase in Y-maze crossing, $F(4,29)=2.95$, $p<0.05$. As the cocaine dose was increased from 0–5 mg/kg, increased activity was observed. No further increases were seen in this strain at the 10 and 15 mg/kg doses. DBA mice exhibited a dose-related increase in activity throughout the dose range used, $F(4,34)=2.86$, $p<0.05$. The C57BL and BALB strains, on the other hand, were more resistant to cocaine's effects on this test. C57BL mice showed an overall effect of cocaine, $F(4,29)=3.30$, $p<0.05$, but significant increases were not seen until the 10 mg/kg

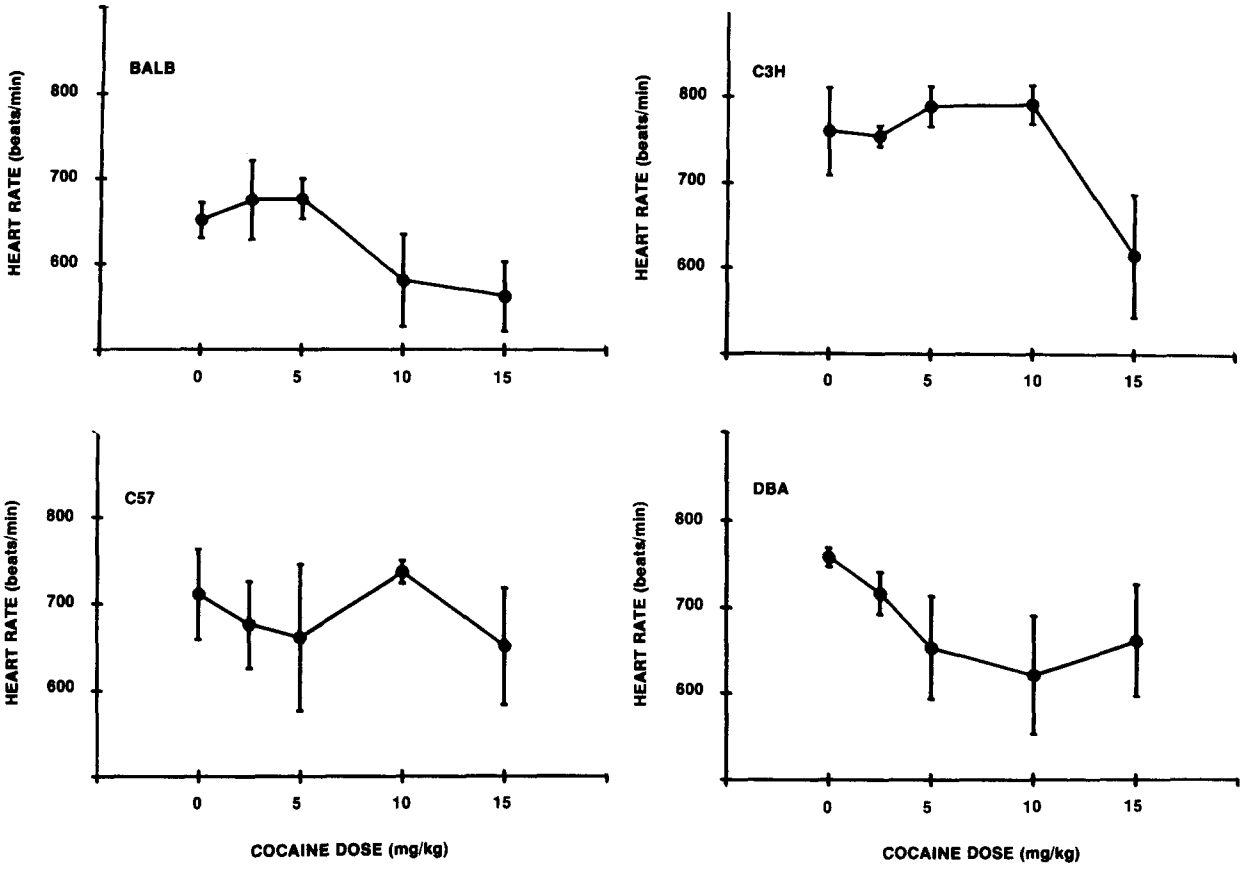

FIG. 3. The dose related effect of cocaine on heart rate of four inbred mouse strains. Cocaine in normal saline was injected IP. After completion of the Y-maze test animals were placed in a restrainer and the heart rate was measured by means of needle electrodes as described in the experimental section. QRS complexes were counted for 6 seconds. Values represent the mean±S.E.M. of observations on 6–8 animals.

dose was attained. Cocaine did not significantly alter Y-maze crosses in the BALB strain at any of the test doses, $F(4,33)=0.28$, $p>0.05$. The two-way ANOVA detected significant influences of strain, $F(3,111)=20.63$, $p<0.01$, dose, $F(4,111)=5.89$, $p<0.01$, and a significant strain by dose interaction, $F(12,111)=14.55$, $p<0.02$. Therefore, the strains were differentially affected by cocaine and the dose-response relationships differed.

Strain differences in the effects of cocaine on Y-maze rearing activity were also detected (Fig. 2). As was the case with the Y-maze crosses, both qualitative and quantitative differences among the strains were detected. Cocaine elicited an overall effect in C3H mice, $F(4,31)=6.35$, $p<0.01$. A dose-dependent increase and then decrease in rearing activity was observed. Rearing increased between 0 and 5 mg/kg and returned to control levels with further increase in cocaine dose. DBA mice showed what appeared to be a gradual increase in rearing activity throughout the entire dose range but the ANOVA indicated no overall drug effect, $F(4,34)=2.12$, $p>0.05$. Similarly, C57BL mice were unaffected by cocaine in this test, $F(4,29)=1.52$, $p>0.05$, whereas BALB mice were depressed by cocaine doses that exceeded 5 mg/kg, $F(4,33)=4.22$, $p<0.01$. These qualitative comparisons are consistent with the results obtained from the two-way ANOVA where significant effects of strain, $F(3,111)=31.11$, $p<0.01$, and dose, $F(4,111)=3.35$, $p<0.02$, were detected. A significant strain by dose interaction term, $F(12,111)=3.61$, $p<0.01$, was also obtained which supports the contention that the strains were differentially affected by increases in cocaine dose.

Because cocaine elicits powerful effects on the cardiovascular system in humans, the effects of these doses of cocaine on heart rate were also measured. The results of these observations are presented in Fig. 3. Once again, strain differences were detected (two-way ANOVA, $F(3,111)=4.71$, $p<0.01$). Cocaine treatment elicited decreases in heart rate in only one of the four strains. The heart rates of C3H mice were unaffected by cocaine until the 15 mg/kg dose was reached at which point a significant depression was seen, $F(4,31)=3.09$, $p<0.05$; *post hoc* analysis indicated that the 15 mg/kg dose was different from all others $p<0.05$). Cocaine did not affect the heart rates of C57BL, $F(4,29)=0.37$, $p>0.05$, BALB, $F(4,33)=1.89$, $p>0.05$, and DBA mice, $F(4,34)=1.11$, $p>0.05$.

In an attempt to ascertain whether these strain differences in response to cocaine are due to pharmacokinetic differences, the concentration of [^{3}H]-cocaine was measured in brain following the injection of 5 and 50 mg/kg cocaine doses. The results of these experiments are presented in Table 1. Strain differences in cocaine content were minimal. The only significant difference found was DBA>C3H at the 50 mg/kg dose ($p<0.05$).

TABLE 1

BRAIN CONCENTRATION OF [^{3}H]-COCAINE IN FOUR INBRED MOUSE STRAINS

Strain	[^{3}H]-Cocaine (nmol/g of tissue)
Brain concentration of unhydrolyzed cocaine 15 minutes after injection with a 5 mg/kg dose	
C3H	1.99 ± 0.42
DBA	1.54 ± 0.06
BALB	2.32 ± 0.36
C57	1.48 ± 0.10
Brain concentrations of unhydrolyzed cocaine 15 minutes after injection with a 50 mg/kg dose	
C3H	45.7 ± 1.00
DBA	53.7 ± 3.10
BALB	38.6 ± 9.50
C57	43.3 ± 4.80

n=3–4.

Only significant difference: DBA > C3H at 50 mg/kg $p<0.05$, Student's t-test.

DISCUSSION

The results presented here clearly demonstrate strain differences in Y-maze crosses and rearing activity and in heart rate as well as in response to cocaine. Interestingly, the relative rank order of the strains varied for the various tests as well as for the cocaine effects. The C3H strain was the most sensitive to cocaine's effects on every measure, but the rank order of the other strains varied for the various measures. For example, in Y-maze crossing activity the BALB strain was the most resistant; cocaine did not alter the activity of BALB mice at any of the test doses whereas a suggestion of an increase in activity was detected in DBA mice. The effects of cocaine on Y-maze rearing activity are complex in that one strain (C3H) showed a biphasic response, two (C57BL and DBA) were unaffected by cocaine, and another (BALB) showed a dose-related decrease. Cocaine elicited statistically significant decreases in heart rate in only one of the strains (C3H). The BALB and DBA strains showed a pattern of cocaine effect on heart rate that resembles the effect seen in the C3H strain but the variance in response was so large in these strains that statistically significant effects were not detected. The strains differed in rank order for baseline activity in the various tests as well as in cocaine response. However, there was no consistent relationship between baseline activity and cocaine effects.

The finding that the four strains vary in their relative sensitivity to the effects of cocaine on the various tests suggests that strain differences in cocaine disposition are not the primary cause of the variance in response. If pharmacokinetic differences were the primary cause of the strain differences, it seems reasonable to expect that a given strain would be uniquely resistant or sensitive to every test. Differences among the strains in brain cocaine content were not detected 15 min after cocaine administration. Therefore, we conclude that the strain differences in response to cocaine are likely due to differences in tissue sensitivity.

Shuster *et al.* [28] have assessed the effects of acute and chronic cocaine injection on locomotor activity in three inbred mouse strains (A, BALB, and C57BL). They reported strain differences in activation following a single dose as well as in sensitization (increased drug effect) following chronic treatment. The least affected strain (A) showed the greatest behavioral sensitization following chronic cocaine injection. Cocaine metabolism was not measured. Nonetheless, the results of this study when combined with our results clearly demonstrate that inbred mouse strains differ in their behavioral responses to cocaine.

Several investigators have used some or all of these mouse strains in studies of the genetic influence on response to other psychoactive agents. For example, Moisset and Welch [22] measured the effect of a 5 mg/kg dose of amphetamine on the open field activity of C57BL/10J (a strain that is very closely related to the C57BL/6Ibg mice that were used in our study) and BALB/cJ mice. These investigators found that amphetamine treatment affected the C57BL mice to a greater degree than it did the BALB mice. Meier *et al.* [20] studied the effects of amphetamine (25 mg/kg) on jiggle cage activity in the same four strains as were used in our study. Only C57BL/6J mice showed an increase in jiggle cage activity. Thus, it appears as though C57BL mice may be uniquely sensitive to the actions of amphetamine whereas they are among the most resistant of the strains that we tested with regard to their responses to cocaine. The finding that the relative sensitivity of genetically-defined mouse strains differs for amphetamines and cocaine suggests differences in genetic regulation of response. An important implication of this finding is that it is not likely that cocaine and amphetamine have identical mechanisms of action.

Strain differences in response to a psychoactive drug can be used as a valuable first step in testing hypotheses concerning the potential mechanism of action of that drug. For example, comparisons of various mouse strains with regard to their sensitivity to ethanol and to nicotine have provided data that support the hypothesis that ethanol works by increasing membrane fluidization [10] and that sensitivity to nicotine-induced seizures is regulated by the number of nicotinic receptors in the hippocampus [21]. It has been suggested that the inhibition of reuptake of brain amines into presynaptic nerve endings may be an important mechanism of action for cocaine [13, 23, 31]. Strain differences in behavioral sensitivity to cocaine may prove useful in testing the hypothesis that cocaine does, indeed, exert its effects via an action on monoamine uptake. If cocaine exerts its actions by affecting monoamine uptake those mouse strains that are most sensitive to behavioral effects of cocaine should also be most sensitive to cocaine's effects on monoamine uptake. Alternatively, if behavioral effects and biochemical effects are not correlated data will have been obtained which will allow rejection of the "cocaine works by inhibiting monoamine reuptake" hypotheses. It should be remembered that adequate testing using the genetic method requires that a sufficiently large (greater than 10) number of inbred strains be used.

In summary, the results presented here clearly demonstrate that genetic factors regulate the response of the mouse to cocaine. Further studies of genetic influence on acute and chronic response to this agent in laboratory animals may ultimately prove useful in explaining why some individuals use and abuse this drug while others do not.

ACKNOWLEDGEMENT

This work was supported, in part, by a grant from the National Institute on Drug Abuse (DA-03194).

REFERENCES

1. Belknap, J. K., S. Waddingham and G. Ondrusek. Barbiturate dependence in mice induced by a single short-term oral procedure. *Physiol Psychol* **1:** 394–396, 1973.
2. Bustos, M. L. and D. B. Hoffman. Detection and identification of cocaine, its metabolites, and its derivatives. In: *Cocaine: Chemical, Histological, Clinical, Social, and Treatment Aspects*, edited by S. J. Mule. Cleveland: CRC, 1976, pp. 35–58.
3. Castellano, C. and A. Oliverio. A genetic analysis of morphine-induced running and analgesia in the mouse. *Psychopharmacologia* **41:** 197–200, 1975.
4. Cloninger, C. R., M. Bohman and S. Sigvardsson. Inheritance of alcohol abuse: Cross-fostering analysis of adopted men. *Arch Gen Psychiatry* **38:** 861–868, 1981.
5. Cloninger, C. R., K. O. Christiansen, T. Reich and I. I. Gottesman. Implications of sex differences in the prevalence of antisocial personality, alcoholism, and criminality for models of familial transmission. *Arch Gen Psychiatry* **35:** 941–951, 1978.
6. Crabbe, J. C. Sensitivity to ethanol in inbred mice: Genotypic correlations among several behavioral responses. *Behav Neurosci* **97:** 280–289, 1983.
7. File, S. E., D. J. Greenblatt, I. L. Martin and C. Brown. Long-lasting anticonvulsant effects of diazepam in different mouse strains: Correlations with brain concentrations and receptor occupancy. *Psychopharmacology (Berlin)* **86:** 137–141, 1985.
8. Fisher, R. A. Cancer and smoking. *Nature* **182:** 596, 1958.
9. Goldstein, A., R. Warren and S. Kaizer. Psychotropic effects of caffeine in man. I. Individual differences in sensitivity to caffeine-induced wakefulness. *J Pharmacol Exp Ther* **149:** 156–159, 1965.
10. Goldstein, D. B., J. H. Chin and R. C. Lyon. Ethanol disordering of spin-labeled mouse brain membranes: Correlation with genetically determined ethanol sensitivity of mice. *Proc Natl Acad Sci USA* **79:** 4231–4233, 1982.
11. Goodwin, D. *Is Alcoholism Hereditary?* New York: Oxford Univ. Press, 1976.
12. Hatchell, P. C. and A. C. Collins. The influence of genotype and sex on behavioral sensitivity to nicotine in mice. *Psychopharmacology (Berlin)* **71:** 45–49, 1980.
13. Heikkila, R. E., F. S. Cabbat and R. C. Duvoisin. Motor activity and rotational behavior after analogs of cocaine: Correlation with dopamine uptake blockade. *Commun Psychopharmacol* **3:** 285–290, 1979.
14. Kakihana, R. Alcohol intoxication and withdrawal in inbred strains of mice: Behavioral and endocrine studies. *Behav Neural Biol* **26:** 97–105, 1979.
15. Khanna, J. M., A. D. Le, A. E. LeBlanc and G. Shah. Initial sensitivity versus acquired tolerance to ethanol in rats selectively bred for ethanol sensitivity. *Psychopharmacology (Berlin)* **86:** 302–306, 1985.
16. Koblin, D. D. and J. E. Deady. Anaesthetic requirements in mice selectively bred for differences in ethanol sensitivity. *Br J Anaesth* **53:** 5–10, 1981.
17. Lapin, I. P. Effects of apomorphine in mice of different strains. In: *Psychopharmacogenetics*, edited by B. E. Eleftheriou. New York: Plenum Press, 1975, pp. 19–33.
18. Marks, M. J., E. Romm, S. Bealer and A. C. Collins. A test battery for measuring nicotine effects in mice. *Pharmacol Biochem Behav* **23:** 325–330, 1985.
19. Marks, M. J., E. Romm, D. K. Gaffney and A. C. Collins. Nicotine-induced tolerance and receptor changes in four mouse strains. *J Pharmacol Exp Ther* **237:** 809–819, 1986.
20. Meier, G. W., J. L. Hatfield and D. P. Foshee. Genetic and behavioral aspects of pharmacologically induced arousal. *Psychopharmacologia* **4:** 81–90, 1963.
21. Miner, L. L., M. J. Marks and A. C. Collins. Genetic analysis of nicotine-induced seizures and hippocampal nicotinic receptors in the mouse. *J Pharmacol Exp Ther* **239:** 853–860, 1986.
22. Moisset, B. and B. L. Welch. Effects of d-amphetamine upon open field behavior in two inbred strains of mice. *Experientia* **29:** 625–626, 1973.
23. Pylatuk, K. L. and J. H. McNeill. The effects of certain drugs on uptake and release of [^{3}H]noradrenaline in rat whole brain homogenates. *Can J Physiol Pharmacol* **54:** 457–468, 1976.
24. Ritz, M. C., F. R. George, C. M. de Fiebre and R. A. Meisch. Genetic differences in the establishment of ethanol as a reinforcer. *Pharmacol Biochem Behav* **24:** 1089–1094, 1986.
25. Seale, T. W., T. H. Roderick, P. Johnson, L. Logan, O. M. Rennert and J. M. Carney. Complex genetic determinants of susceptibility to methylxanthine-induced locomotor activity changes. *Pharmacol Biochem Behav* **24:** 1333–1341, 1986.
26. Shields, J. *Monozygotic Twins Brought Up Apart or Brought Up Together.* London: Oxford Univ. Press, 1962.
27. Shuster, L. Genetic analysis of morphine effects: Activity, analgesia, tolerance and sensitization. In: *Psychopharmacogenetics*, edited by B. E. Eleftheriou. New York: Plenum Press, 1975, pp. 73–97.
28. Shuster, L., G. Yu and A. Bates. Sensitization to cocaine stimulation in mice. *Psychopharmacology (Berlin)* **52:** 185–190, 1977.
29. Spuhler, K., B. Hoffer, N. Weiner and M. Palmer. Evidence for genetic correlation of hypnotic effects and cerebellar purkinje neuron depression in response to ethanol in mice. *Pharmacol Biochem Behav* **17:** 569–578, 1982.
30. Victor, B. S., M. Lubetsky and J. F. Sreden. Somatic manifestations of caffeinism. *J Clin Psychiatry* **42:** 185–188, 1981.
31. Yu, J. H. and C. B. Smith. Effect of cocaine and desmethylimipramine on the uptake, retention and metabolism of ^{3}H-5-hydroxytryptamine in rat brain slices. *Pharmacology* **15:** 242–253, 1977.