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Cocaine and Seizure Protection in Mice of Varying Brain Weights[1]

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DONOVICK, P. J., R. G. BURRIGHT, R. J. FANELLI, B. SYMCHOWICZ AND A. RITZ. *Cocaine and seizure protection in mice of varying brain weights.* PHARMAC. BIOCHEM. BEHAV. **14**(3) 409–414, 1981.—The effects of cocaine administration on seizure duration and proportion of animals that seized were examined in mice genetically selected for differential brain weight. In the two experiments reported, mice of the Fuller brain weight lines and of the Binghamton Heterogeneous stock were used. In Experiment 1, 21 day old male and female mice were administered either 0, 5, 10 or 20 mg/kg of cocaine hydrochloride. Fifteen minutes after each mouse was injected, a transcorneal shock was administered to that animal. Type and duration of seizure were measured. Low brain weight mice were more susceptible to seizures and showed more severe seizures than the medium and high brain weight mice or the Heterogeneous stock. Cocaine hydrochloride provided a degree of protection against seizures in a dose dependent fashion, particularly in the medium brain weight and Heterogeneous stock mice. Experiment 2 examined whether repeated daily doses of cocaine (15–21 days of age) would alter the pattern of susceptibility described above. In this experiment mice were given either 0 mg/kg or 10 mg/kg of cocaine hydrochloride. At 21 days of age, 15 minutes after injection, mice were subjected to transcorneal shock and the subsequent nature and duration of seizures observed. Mice of the low brain weight line were again seen to have more seizures than those from the high brain weight line. Cocaine decreased the severity of transcorneal induced seizures.

Cocaine Brain weight Seizure susceptibility

AS indicated by a recent symposium [11], there have been a number of attempts to relate genetically based differences in brain weight to nervous system function and behavior. Behavioral investigations have shown that mice genetically selected for high brain weights were more active and performed better in learning tasks than mice selected for low brain weights [5,22]. Using an independent selection regime, Fuller found that brain weight (BW) was related to open field activity, rotorod performance, age of eye opening and edge avoidance [7]. While Jensen [13] failed to find evidence for major differences in learning capability of these Fuller BW lines, both he, and Ahroon and Fuller [1] have pointed to the critical aspect of differential sensitivity to test conditions in these lines. Further, although initial learning may not vary among these lines, retention may indeed be altered by brain weight [12]. Other indications that the brain weight lines may be differentially sensitive to environmental factors are suggested by the measurements of agonistic behavior [8,10]. These lines may also vary in their sensitivity to neonatal administration of thyroxine [2]. Thus, these lines may be differentially sensitive to their external and internal environments. It is not surprising then that Fuller [6] recently found differences in the three brain weight lines regarding their susceptibility to audiogenic seizures. Following priming, the high and mid brain weight lines were more sensitive than the low brain weight lines to auditory stimulation, especially around 21 days of age.

Seizure susceptibility, produced by a variety of methods, has been used as a measure of cortical sensitivity and as an indirect method of assessing an animal's general responsivity to stimuli (e.g., [16]). The use of electroconvulsive shock has a long history, both in research concerned with mechanisms of memory (typically using rodents) and a therapeutic technique in humans. Unfortunately, little is known concerning genetic predispositions to such seizures. The effects of pharmacological agents such as ethanol [17] and therapeutic drugs [4,19] on the initiation and severity of seizures have been examined. Chronic administration of cocaine has been shown to facilitate kindled seizures in adult rats [15,20] and monkeys [18]. However, cocaine has been shown to provide protection against flurothyl-induced seizures in young mice [9]. Interestingly this effect was reversed in older animals [9]. We examined the impact of cocaine administration on seizure susceptibility of the Fuller brain weight lines which differ in norepinephrine and dopamine [3].

Fuller found [6] maximum differential sensitivity to audi-

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TABLE 1
SEIZURE SUSCEPTIBILITY

		Male				Female			
Cocaine dose*		0	5	10	20	0	5	10	20
HET	Proportion seizing	9/11	8/10	4/10	1/10	8/10	5/10	6/10	3/10
	Median duration seizure†	12	11	(11.5)	(5)	15	10	14	(7)
H	Proportion seizing	3/10	3/10	2/10	1/ 9	1/10	2/10	4/10	1/10
	Median duration seizure	(7)	(17)	(4)	(11)	(5)	(7.5)	(12)	(13)
M	Proportion seizing	5/10	5/10	4/10	1/10	6/11	8/10	6/10	5/10
	Median duration seizure	15	7	(8)	(5)	21	7.5	10	7
L	Proportion seizing	9/10	7/10	8/10	9/10	9/10	10/10	8/12	7/10
	Median duration seizure	20	17	10.5	5	19	12.5	12	22

*mg/kg of Cocaine Hydrochloride, or equal volume saline (0) injections.
†Median duration (sec) of seizure based on those animals displaying seizures; note that durations in parentheses are based on fewer than 50% of the group.

ogenic seizures between lines around 21 days of age. We used this age for examining transcorneal ECS. In Experiment 1 we examined the effects of a single injection of cocaine HCl (0, 5, 10, or 20 mg/kg) on transcorneally-induced electroconvulsive seizures in the Fuller BWS and HET stock mice. Experiment 2 examined how repeated administration of cocaine over a developmentally sensitive period (15–21 days post partum) of the mouse's life alters seizure susceptibility at 21 days of age.

EXPERIMENT 1

METHOD

Subjects were male and female offspring of the three Fuller brain weight lines (high—HBW; medium—MBW; and low—LBW) and the Binghamton Heterogeneous (HET) stock [7]. All mice were derived from matings in our laboratory. Mating pairs were maintained in transparent, plastic cages with access to Charles River mouse chow and water ad lib. Both parents were continuously maintained in the same cage (and thus) up until the time of drug administration. At birth, litters were culled to a maximum of 4 males and 4 females wherever possible. The vivarium in which mice were housed was maintained at 22°C±1°C with white lights on between 8:00 a.m. and 8:00 p.m.; red lights were on between 8:00 p.m. and 8:00 a.m.

When 21 days of age, male and female pups of the three brain weight lines and the HET strain were taken from their home cages, weighed and assigned to receive either 0, 5, 10, or 21 mg/kg of cocaine hydrochloride. Mice from each litter were distributed among all four drug groups with approximately equal numbers of males and females in each group. A total of 323 mice were employed in this experiment, resulting in approximately 10 males and 10 females from each of the four strains being assigned to each drug group.

Injections were given between 9:00 and 11:00 a.m. with a drug solution which was made up weekly. The experimenter was "blind" relative to drug condition. Fifteen minutes after intraperitoneal drug administration the pups were subjected to transcorneal, electroconvulsive shock (ECS) administered by a Lafayette Electroconvulsive Shock apparatus (Model A615B). The corneal electrodes were supplied by Dr. Symchowicz of the Schering Corporation. The electrodes were lightly coated with Hewlett-Packard redux creme and 750 volts were passed through the mouse and a 180k ohm resistor for 0.5 sec, resulting in approximately a 4 mA shock.

Immediately following delivery of transcorneal shock the mouse was placed in a transparent cage and the duration and severity of the seizure was recorded. Since all animals that seized did so almost instantly, it was not possible to record latency measures between shock termination and onset of seizure. We measured the latency between the onset of the shock and termination of any seizure activity observed, whether tonic or clonic. All animals were then sacrificed. Although brain weights were not measured in these experiments, routine analyses have shown that line differences are now quite stable (e.g., [8]). The most recent measurement of the brain weights of the selected lines was done using mice from just one and two generations prior to those mice used in

the present study. At 21 days of age, the whole-brain, wet-tissue weights expressed as mean mg tissue, with standard errors, were: HBW=470.7±5.1; MBW=406.4±3.1; and LBW=388.2±2.7 [3]. Whole brain, wet tissue weights for 21 day old HET mice were 433.0±6.0.

RESULTS AND DISCUSSION

Table 1 provides both the proportion of 21-day-old mice that seized and the median duration (in seconds) of those animals that seized for each strain, dosage and sex group employed. Of the 323 animals observed, 168 (52%) showed signs of seizure and only 8 of those mice died (all in 0 dose groups); no MBW mice died. The proportion data show that given only a saline injection (0 dose), the HET and LBW mice, regardless of sex, displayed a high degree of seizure susceptibility to the transcorneal ECS: i.e., 80–90% of the animals in those groups seized. In contrast, mice from the MBW line were intermediate in susceptibility (50–55%) and the HBW line was least susceptible (10–30%). Those groups given a single injection of cocaine 15 minutes prior to ECS administration generally appeared less susceptible to seizures than their control counterparts in terms of the proportion data. However, while the HET mice displayed a clear dose-response function in this regard, the LBW animals remained highly susceptible to ECS regardless of drug dose. The MBW mice, especially males, also showed a decrease in seizure susceptibility with increasing cocaine dose. However, protection from seizure as a result of cocaine administration was not as apparent in the least susceptible HBW groups.

For purposes of statistical analysis the frequencies of observed seizure (among type) vs. no seizure (this was nearly a median-split on duration since the overall median duration, including no seizure, was 3 sec) were cast into a four dimensional contingency table (strain, dose, sex, seizure), providing an overall $\chi^2=97.36$, $df=31$, $p<0.001$. Subsequent partitioning (cf.[23]) indicated that the strain × seizure and dose × seizure components were statistically significant ($\chi^2=58.80$, $df=3$, $p<0.001$; $\chi^2=13.39$, $df=3$, $p<0.001$, respectively) and together accounted for almost 74% of the overall χ^2 value. While none of the remaining χ^2 values associated with the partitioning reached statistical significance, the strain × dose× seizure component (9 *df*) represented more than 11% of the overall χ^2 value; and the strain × sex× dose × seizure (9 *df*) source represented more than 8% of the overall χ^2 value. Thus, the apparent differential line (and sex) effects of the drug dose on proportion of seizure occurrence should not be totally discounted.

Examination of the duration of seizure data for the 168 mice that did seize generally appear to support and supplement the frequency of seizure results described above. However, it is important to keep in mind that different numbers of animals seized in each group and thus interpretation of these duration data must be cautious. Median seizure duration typically decreased with increased cocaine dosage, especially in the HET and MBW mice, regardless of sex. This reduction in duration of seizure was also apparent in the LBW males and to a lesser degree in the LBW females. The median duration data from the seizure resistant HBW mice is least convincing in this respect, but is based upon very few animals per group.

As can be seen in Table 2, significant strain differences in body weight existed; on the average, at weaning, the HBW mice were the heaviest and LBW mice the lightest animals tested. However, the range of body weights overlapped extensively (e.g., LBW male 6.0–14.8; HBW male 7.9–17.0), and bodyweights within strains did not clearly predict either seizure or its duration.

TABLE 2

MEAN BODYWEIGHT IN GRAMS (EXPERIMENT 1) WITH RANGE IN PARENTHESIS

	Male	Female
HET	12.0 (6.9–15.0)	11.8 (8.2–14.8)
HBW	12.3 (8.0–17.0)	12.5 (6.8–18.9)
MBW	12.1 (8.5–15.9)	11.2 (8.2–14.3)
LBW	10.5 (6.0–14.8)	10.2 (4.3–13.3)

Experiment 2 examined potential shifts in the effects of repeated administration of 10 mg/kg cocaine hydrochloride. Daily injections were given to the BW lines and HET mice from the time they were 15 until they were 21 days of age.

EXPERIMENT 2

METHOD

General conditions were basically the same as in Experiment 1. When the pups were 15 days of age, the litters were further culled from 8 pups to 4 pups with 2 males and 2 females wherever possible. The 155 pups from the four genomes used in this study were then assigned to either the drug or control group. One male and one female pup from each litter received daily injections (IP) of either 10 mg/kg of cocaine hydrochloride or an equal volume of 0.9% saline solution. For each of 6 consecutive days (ages 15–20 days) the pups were removed from the litter, weighed, injected and returned to the litter. Injections were given between 9:00 and 11:00 a.m. Fifteen minutes after drug administration on the 7th day (age 21) the pups were subjected to transcorneal electroconvulsive shock as described in Experiment 1. The presence of any observable seizure activity and its duration were again recorded; resultant data were treated similar to that in Experiment 1.

Data again were examined in terms of: (a) the proportion of animals that seized in each group and (b) the duration of seizure. As in Experiment 1, these two approaches tended to supplement and support each other. For statistical purposes, a median-split based upon seizure duration (including no seizure = 0 sec duration) was used to divide animals into no or "mild" seizures (below the median) or "more severe" seizures (longer than the median).

RESULTS AND DISCUSSION

Figure 1 illustrates the median seizure duration of all mice which showed signs of seizures separately by line, sex, and drug. Immediately above each bar is the proportion of animals in that group that had a seizure and the mean body weight of animals of that group. Only 2 of the 103 mice that showed signs of seizure died; the duration of their seizures were assigned a value of 120 seconds. Overall, 66% of the 155 mice used in Experiment 1 showed signs of seizures. As in Experiment 1, the LBW line had proportionally the most animals seizing and the HBWs the least. The HET and MBW mice were intermediate in this respect. Again, differences in

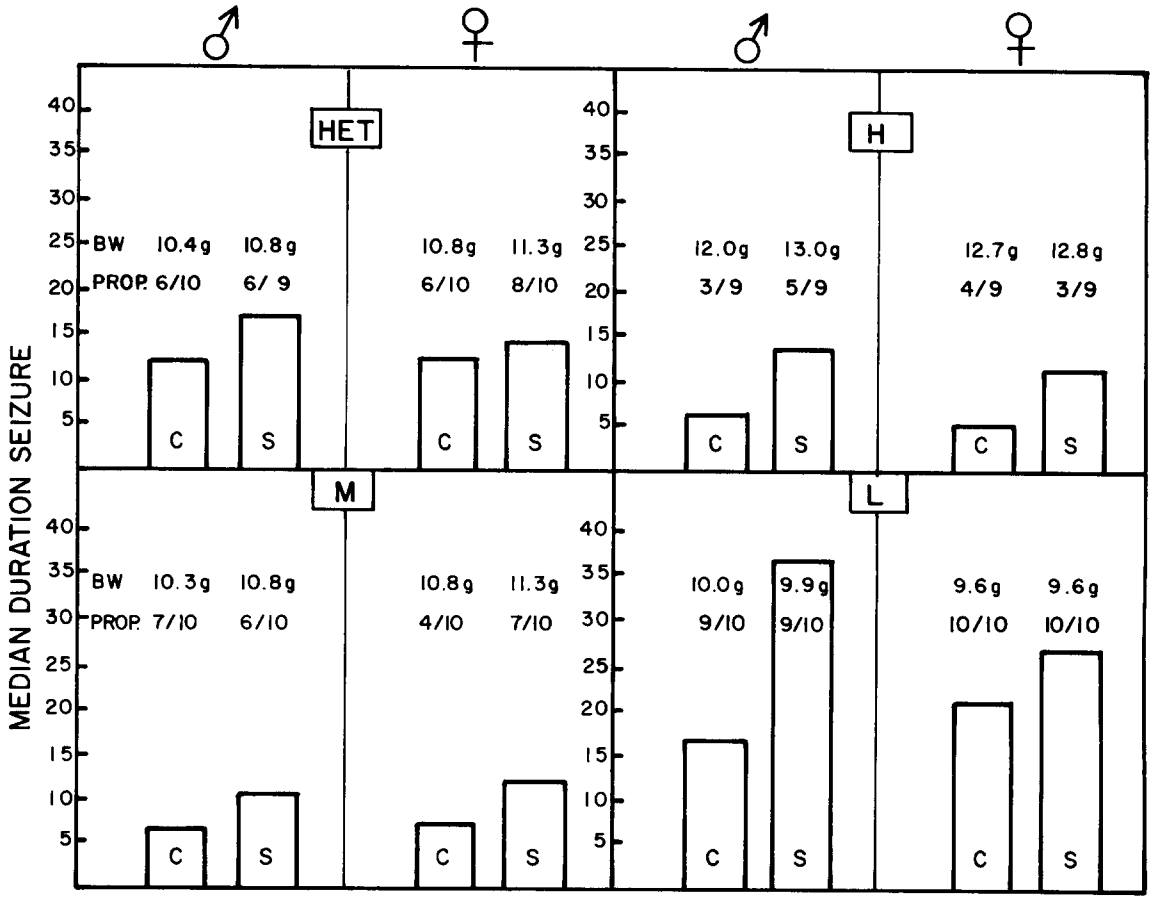

FIG. 1. A representation of the median duration of seizures of those animals of each strain and sex that exhibited a transcorneally induced electroconvulsive seizure. This figure also contains the median body weight and the proportion of animals of each group that had a seizure. C=cocaine, S=saline.

body weight did not seem to predict either which mice would exhibit seizures nor the median duration of the seizure for those animals which had seizures.

For purposes of statistical analysis, since 66% of the mice in this experiment showed signs of seizure, all mice (including those which showed no seizure) were ranked on the basis of duration of seizures and divided in half. Thus, similar to the seizure vs. no seizure analysis conducted in Experiment 1, the lowest group included 78 mice whose seizures ranged from 0–8.3 seconds in duration. The upper half (77 mice) had "more severe" seizures which ranged from 8.5 sec to 73 sec (or death). As might be expected from data presented in Fig. 1, the overall χ^2 value derived from the resulting median-split contingency table was significant ($\chi^2=45.64$, $df=15$, $p<0.001$). Subsequent partitioning of the degrees of freedom (cf.[28]) revealed that nearly 82% of the overall χ^2 value was accounted for by the strain × severity component ($\chi^2=37.3$, $df=3, p<0.001$). Thus, most LBW mice had relatively severe seizures, whereas only a few HBW mice fell into that category—the HET and MBW mice were intermediate in this respect. In addition, the drug × severity component showed that only 41% of the mice repeatedly administered 10 mg/kg cocaine had seizures classified as relatively severe, whereas 58% of the seizures observed in repeated saline injection control mice were of relatively long duration ($\chi^2=$ 4.70, $df=1, p<0.05$). Since so many (95%) of the LBW mice showed some seizure as a result of the transcorneal shock, any "protection" afforded by cocaine could *only* be expressed in terms of relative duration of the seizures observed. In fact, such "protection" by cocaine is suggested in the median seizure duration values for LBW mice that seized

TABLE 3

COMPARISONS OF MEDIAN ECS-INDUCED SEIZURE DURATIONS (SEC) FOR SINGLE (EXPERIMENT 1) AND REPEATED (EXPERIMENT 2) INJECTION CONDITIONS

	10 mg/kg Cocaine			
	Repeated-Single			
	Male	Female	Male	Female
HET	+5	0	+0.5	−1.0
HBW	+6	+7	+3.0	−6.0
MBW	−4	−7	−2.0	−1.0
LBW	+16	+8	+6.5	+8.0

(Fig. 1), and is clearest for males (Median duration = 16 seconds for cocaine and 36 seconds for saline groups) of this strain. A partitioning of the Kruskal-Wallis H-statistic [21] using ranked seizure durations only for LBW mice showed the drug condition statistically significant ($H = 5.35$, $df = 1$, $p < 0.025$; median cocaine = 18 seconds, median saline = 28 seconds).

GENERAL DISCUSSION

In summary, the brain weight lines differed in their duration of transcorneally-induced ECS seizures as well as the proportion of animals that seized in both experiments, and LBW line mice seized more often than mice of the other brain weight lines or the HET stock. Further, in general, cocaine administration provided some protection against seizures.

Although different numbers of animals between groups are represented in the duration of seizure data, comparison of the median durations for the single and repeated dose experiments suggests some interesting problems. Table 3 presents a comparison of the average duration of seizure of comparable groups for the two experiments. Positive scores indicate that the duration of seizures was, on the average, longer for animals with repeated doses. For instance, male HET mice given repeated saline injections had a median seizure which was 5 sec longer than comparable animals given a single dose of saline. For the saline groups, seizure duration of those mice which seized after repeated injections tended to be longer than those seizures seen after single injections in all strain/sex groups except for the MBW mice. When we compared the 10 mg/kg cocaine groups, repeated administration generally decreased duration of seizure in all female groups except for LBW mice and increased duration of all male groups except the MBW. Thus, compared with single injection (Experiment 1) groups, repeated injections of either saline or 10 mg/kg cocaine tended to decrease duration of seizure in all MBW groups, yet increased seizure durations in all LBW groups. The differential sensitivity implied by these data indicate the difficulties in choosing an appropriate baseline group for comparison when, for example, repeated injections are employed.

While our results support the general notion that the brain weight lines differ in their susceptibility to seizures, they are in contrast to the data reported by Fuller [6] where the HBW mice (following priming) were most sensitive to audiogenically induced seizures. Such results indicate different central mechanisms for the two forms of seizure, which is not surprising. Similarly, cocaine administration may have differential effects depending on mode of seizure induction and perhaps stage of development (compare our data, [9, 15, 18]). Although our data in themselves do not define or explain the nature of differences in the brain weight lines, they do add to our growing understanding of these mice (e.g. [11]). Certainly, brain weight differences among these lines, selected specifically for that phenotype, are not the only characteristics upon which such lines may differ; thus, the data reported here can only suggest that within as well as between species differences in brain weight may be important in some aspects of the organization of the central nervous system and behavior [6,14].

REFERENCES

1. Ahroon, J. K. and J. L. Fuller. Performance characteristics of maze learning in mice selected for high and low brain weight. *J. comp. physiol. Psychol.* **90:** 1184–1190, 1976.
2. Chen, C.-S. and J. L. Fuller. Neonatal thyroxine administration, behavioral maturation, and brain growth in mice of different brain weight. *Devl Psychobiol.* **8:** 355–361, 1975.
3. Dudek, B. C. and P. J. Berman. Biochemical correlates of selective breeding for brain size. In: *Development and Evolution of Brain Size: Behavioral Implications*, edited by M. R. Hahn, C. Jensen and B. C. Dudek. New York: Academic Press, 1979.
4. Edmonds, H. L., L. G. Stark, D. M. Stark, C. R. McCormack, D. M. Sylvester and S. I. Bellin. The anticonvulsant activity of ropizine in the rat. *J. Pharmac. exp. Ther.* **208:** 236–242, 1979.
5. Elias, M. F. Differences in spatial discrimination reversal learning for mice genetically selected for high brain weight and unselected controls. *Percept. Mot. Skills* **28:** 707–712, 1969.
6. Fuller, J. L. Fuller BWS lines: History and results. In: *Development and Evolution of Brain Size: Behavioral Implications*, edited by M. E. Hahn, C. Jensen and B. C. Dudek. New York: Academic Press, 1979.
7. Fuller, J. L. and B. H. Herman. Effect of genotype and practice upon behavioral development in mice. *Devl Psychobiol.* **7:** 21–30, 1974.
8. Gonsiorek, J. C., P. J. Donovick, R. G. Burright and J. L. Fuller. Aggression in low and high brainweight mice following septal lesions. *Physiol. Behav.* **12:** 813–818, 1974.
9. Greer, C. A. and H. P. Alpern. Maturational changes related to dopamine in the effects of d-amphetamine, cocaine, nicotine, and strychnine on seizure susceptibility. *Psychopharmacology* **64:** 255–260, 1979.
10. Hahn, M. E., S. B. Haber and J. L. Fuller. Differential agonistic behavior in mice selected for brain weight. *Physiol. Behav.* **10:** 759–762, 1973.
11. Hahn, M. E., C. Jensen and B. C. Dudek. *Development and Evolution of Brain Size: Behavioral Implications*, edited by M. R. Hahn, C. Jensen and B. C. Dudek. New York: Academic Press, 1979.
12. Herman, B. H. and Z. M. Nagy. Development of learning and memory in mice genetically selected for differences in brain weight. *Devl Psychobiol.* **10:** 65–75, 1977.
13. Jensen, C. Generality of learning differences in brain-weight-selected mice. *J. comp. physiol. Psychol.* **91:** 629–641, 1977.
14. Jerison, H. J. The evolution of diversity in brain size. In: *Development and Evolution of Brain Size: Behavioral Implications*, edited by M. E. Hahn, C. Jensen and B. C. Dudek. New York: Academic Press, 1979.
15. Kilbey, M. M., E. H. Ellinwood and M. E. Easler. The effects of chronic cocaine pretreatment on kindled seizures and behavioral stereotypies. *Expl Neurol.* **64:** 306–314, 1979.
16. McCarty, R. and C. H. Southwick. The development of convulsive seizures in the grasshopper mouse (*Onychomys torridus*). *Devl Psychobiol.* **8:** 547–552, 1975.
17. Mucha, R. F. and J. P. J. Pinel. Increased susceptibility to kindled seizures in rats following a single injection of alcohol. *J. stud. Alcohol* **40:** 258–271, 1979.
18. Post, R. M., R. T. Kopanda and K. E. Black. Progressive effects of cocaine on behavior and central amine metabolism in rhesus monkeys: Relationship to kindling and psychosis. *Biol. Psychiat.* **11:** 403–419, 1976.

19. Racine, R., W. M. Burnham and K. Livingston. The effect of procaine hydrochloride and diazepam, separately or in combination, on cortico-generalized kindled seizures. *Electroenceph. clin. Neurophysiol.* **47:** 204–212, 1979.
20. Rackham, A. and R. A. Wise. Independence of cocaine sensitization and amygdaloid kindling in the rat. *Physiol. Behav.* **22:** 631–633, 1979.
21. Scheirer, C. J., W. S. Ray and N. Hare. The analysis of ranked data derived from completly randomized factorial designs. *Biometrics* **32:** 429–434, 1976.
22. Wimer, C., T. H. Roderick and R. E. Wimer. Supplementary report: Behavioral differences in mice genetically selected for brain weight. *Psychol. Rep.* **25:** 363–368, 1969.
23. Winer, B. J. *Statistical Principles in Experimental Design*. New York: McGraw-Hill, 1962.