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Cocaine Reward and Locomotor Activity in C57BL/6J and 129/SvJ Inbred Mice and Their F1 Cross

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MINER, L. L. *Cocaine reward and locomotor activity in C57BL/6J and 129/SvJ inbred mice and their F1 cross.* PHARMACOL BIOCHEM BEHAV **58**(1) 25–30, 1997.—Large individual differences exist among mice in their behavioral responses to drugs of abuse, and many of these differences have a substantial genetic basis. The creation of new animal models using recombinant DNA technology has provided new genetic tools for assessing the role of specific candidate genes in drug response. This study presents a characterization of cocaine activation and reward in the two strains used most commonly for production of knockout mice, C57BL/6J and 129/SvJ, and their outcrossed F1 offspring. Using conditioned place preference, the study demonstrates that there are large strain differences in spontaneous locomotor activity and in the rewarding effects of cocaine. The 129/SvJ strain is hypoactive and is very sensitive to the locomotor activating effects of cocaine but does not develop cocaine-conditioned place preference under conditions that yield significant place preference in C57BL/6J mice. These phenotypes are not inherited in a simple additive manner, but rather the F1 generation resembles the C57BL/6J progenitor strain for a number of the behaviors examined. © 1997 Elsevier Science Inc.

Cocaine Conditioned place preference Locomotor activity Genetics Transgenics

WITH the recent advances in homologous recombination/gene "knockout" technologies, a number of interesting new mouse strains have been produced targeting the expression of specific candidate genes thought to be involved in mediating the psychomotor stimulant effects of many of the drugs of abuse. Recently deleted candidate genes include four subtypes of dopamine receptors, D1a (7,23), D2 (3,10), D3 (2,22), and D4 (10), as well as the primary site of action of cocaine's reward and reinforcing effects, the dopamine transporter (9). Other mouse mutants of interest to neuropsychopharmacologists include targeted deletions of the serotonin 1b receptor (16) and the β2 subunit of the neuronal nicotinic receptor (13), as well as β-endorphin (11), protein kinase C (1), and CAM kinase (20).

In assessing the behavioral effects of such genetic manipulations, it is important to consider the background strains that went into development of the animals. In general, most of the knockout strains have been derived from a C57BL/6J blastocyst that has been injected with transformed embryonic stem cells derived from the 129/SvJ strain (19,21). Most commonly, studies of these mutants are performed on animals generated from the cross of heterozygous mice from an F2 population such that homozygous mice are often compared with wild-type and/or heterozygous littermates that have a mixed genetic background. If an interesting phenotype is identified, it then becomes essential to test a large number of mutants to reliably assess an altered phenotype in a polygenic system.

Pharmacogenetic studies have established that genetic factors are important in mediating many of the physiological, behavioral, and hedonic effects of drugs of abuse (5,6,12). These studies have characterized drug responses in many inbred mouse strains, with the C57BL/6J strain being one of the most thoroughly studied of all inbred strains (17,18). In contrast, the 129/SvJ strain has been used little and is poorly characterized, especially as to its sensitivity to drugs of abuse.

The present study represents the first behavioral characterization of the 129/SvJ strain and its sensitivity to the psy-

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chomotor stimulant effects of cocaine; results are contrasted with observations in the C57BL/6J strain and in F1 animals derived from the mating of the two strains. Spontaneous locomotor activity as well as short-term and long-term habituation, forms of nonassociative learning (14,15), were also determined in these genotypes. By using the conditioned place preference test, the most widely used murine model of drug reward (4), sensitivity to cocaine was assessed by examining the locomotor activating effects and the hedonic effects of the drug after acute administration. The results should be of paramount interest to those investigators seeking to characterize cocaine response in many of the newly developed "knockout" strains.

METHODS

Animals and Drugs

C57BL/6J and 129/SvJ were obtained from Jackson Laboratories (Bar Harbor, ME, USA) and maintained in the animal colonies of the Molecular Genetics Section of NIDA/Division of Intramural Research (Baltimore, MD, USA). Animals were housed in groups of generally five or six animals per cage; food and water were freely available, and the mice were maintained on a 12 L:12 D cycle (lights on at 0700 h), in accordance with guidelines of the American Association for Laboratory Animal Care. All testing was conducted during the animal's light cycle, and animals were housed in our facilities for a minimum of 2 weeks prior to testing. C57BL × 129 F1 animals were bred in our facilities from matings of 129/SvJ males and C57BL/6J females. In the present study, only male mice 55–90 days of age were used for behavioral testing.

Cocaine hydrochloride was dissolved in saline and injected intraperitoneally (IP) in a volume of 10 μl per gram of body weight.

Locomotor Activity Testing

Spontaneous locomotor activity was measured by using Columbus Instruments (Columbus, OH, USA) Opto-Varimex activity monitors linked to an IBM PC/AT computer. Monitors were placed in sound-attenuating chambers, and testing was conducted under dim ambient light conditions. For spontaneous activity levels and habituation studies, individual mice were placed in 46 × 25 × 19-cm (length × width × height) clear plastic cages for 30 min and total distance traveled was calculated from infrared beam breaks. Short-term habituation was assessed over the 30-min time span by determining distance traveled at 5-min intervals. Long-term habituation was assessed over a 2-week time period, testing each of the animals on four nonconsecutive days (Monday, Thursday, Monday, Thursday).

Effects of cocaine on locomotor activity were assessed in mice that had not been exposed to the testing monitors previously. Mice were injected with the indicated doses and placed immediately in the testing environment; then, distance traveled was monitored for 30 min.

Conditioned Place Preference

Conditioned place preference was assessed in Plexiglas chambers that were divided into two compartments. Both internal compartments were 18 × 18 × 18 cm, with one compartment having a wire mesh floor mounted over Plexiglas and the other having corn cob bedding covering the floor. The two unique compartments were separated by a removable Plexiglas wall that, for pre- and postconditioning test sessions, had a 5-cm opening allowing access to both compartments. During the conditioning sessions, the removable wall was inverted, which eliminated the opening and thereby limited access to one compartment. Chambers were placed in Opto-Varimex activity monitors, and the time spent in each compartment was recorded. Conditioned place preferences were assessed by determination of chamber side preference in three phases. Initial preference was determined as the compartment in which a mouse spent more than 450 s out of a 15-min (900-s) trial. Conditioning was conducted over a 4-day period in which cocaine was administered when the animal was restricted for 30 min to the initially nonpreferred compartment and saline was administered when the animal was restricted for 30 min to the preferred compartment. Animals received one conditioning session per day, counterbalanced between saline and cocaine, for a total of two saline pairings and two cocaine pairings. Conditioned place preference assessments followed the last conditioning session by 24 h and involved evaluation of differences in time spent in the drug-paired compartment vs. preconditioning times for that compartment.

Data Analysis

All data were analyzed using Statview Software (Abacus Concepts, Inc., Berkeley, CA, USA). Spontaneous locomotor activity was compared among the genotypes by one-way analysis of variance (ANOVA), and cocaine dose–effects differences were analyzed by two-way (genotype × dose) ANOVA. Repeated-measures ANOVA was used to assess development of habituation. For long-term habituation, significant main effects of strain [$F(2, 17) = 10.24$, $p < 0.01$] and trial [$F(3, 51) = 3.31$, $p < 0.05$], as well as strain × trial interaction [$F(6, 51) = 2.73$, $p < 0.05$], were found. Given the significant interaction effect, each strain was then statistically analyzed separately by repeated-measures ANOVA to determine if significant habituation was developing for the strain. Conditioned place preference data were analyzed by two-way ANOVA (strain × dose). The C57BL/6J, 129/SvJ, and F1 genotypes were compared at the 5-mg/kg cocaine conditioning dose by one-way ANOVA (genotype). Preference was defined as a significant increase in the percentage of time spent in the drug-paired chamber after each conditioning dose.

RESULTS

Spontaneous Locomotor Activity and Development of Habituation

The C57BL/6J strain and the C57 × 129 F1 animals are significantly more active than the 129/SvJ strain, with three times the total distance traveled in a 30-min period (Fig. 1A) [$F(2, 20) = 40.02$, $p < 0.0001$; Bonferroni–Dunn post hoc, $p < 0.0001$]. Because F1 mice are heterozygous at all loci for which their progenitor strains differ, their response is determined by both additive genetic effects and the nonadditive effects of dominance and genic interaction. If the genes influencing locomotor activity act in a purely additive fashion, F1 activity levels should fall midway between the two parental strains. The degree to which the F1 generation deviates from this expected mid-parent value is a measure of dominance (8). The almost identical spontaneous locomotor activity levels between the C57BL/6J and F1 animals indicate complete dominance toward greater activity.

The development of two types of habituation was examined: a) short-term habituation, for which locomotor activity levels were sampled at 5-min intervals (Fig. 1B) during their

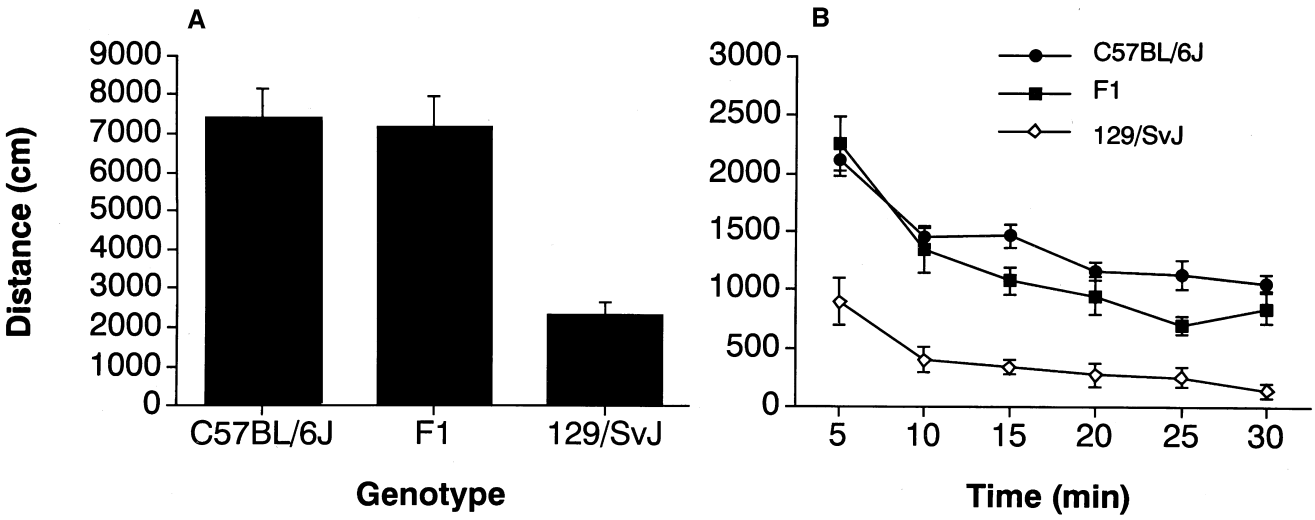

FIG. 1. (A) Spontaneous locomotor activity in C57BL/6J and 129/SvJ inbred mice and their F1 cross. Each bar represents the average (±SEM) distance traveled in a 30-min period for 6–10 mice. (B) Short-term habituation in C57BL/6J and 129/SvJ inbred mice and their F1 cross. Total distance traveled was determined at 5-min intervals during the 30-min testing period. Each point represents the average distance traveled in that period ± SEM for 6–10 mice.

initial exposure to the testing apparatus, and b) long-term habituation, in which mice were repeatedly exposed to the test environment over a 2-week period (Fig. 2). All three genotypes develop significant short-term habituation, as indicated by significant decreases in total distance traveled [ANOVA, time main effect: $F(5, 100) = 54.00$, $p < 0.0001$]. Significant strain differences were also observed [ANOVA, genotype main effect: $F(2, 100) = 40.02$, $p < 0.0001$]. The C57BL/6J and F1 strains have significantly higher activity levels at all time points than the 129/SvJ strain [Bonferroni–Dunn post hoc: $p < 0.0001$] no significant differences were observed between the C57BL/6J and F1 groups. A significant strain × time interaction effect [$F(10, 100) = 2.66$, $p < 0.01$] indicates that the distance traveled by the F1 and C57BL/6J strains decreases more rapidly than for the 129/SvJ strain. This is likely due to a floor effect for the 129/SvJ strain. If only the first two time points are examined, all three strains show rapid decreases in distance traveled that account for most of the total decrease observed.

Examination of the long-term memory processes reflected in the assessment of long-term habituation (Fig. 2) indicates that neither of the two parental strains, C57BL/6J and 129/SvJ, have a diminution of locomotor activity upon repeated exposure to the testing environment. However, the F1 generation does habituate to the testing environment, showing a significant decrease in total distance traveled upon repeated exposure [$F(3, 15) = 8.90$, $p < 0.01$].

Cocaine and Locomotor Activity

All three genotypes demonstrate dose-dependent increases in locomotor activity after cocaine injection (Fig. 3) [two-way ANOVA: $F(3, 76) = 104.97$, $p < 0.0001$]. There are, however, no differences in sensitivity to the locomotor activating effects of cocaine among the three genotypes [$F(2, 76) = 0.552$, $p = 0.58$]. Interestingly, the activity measured after saline injection of the 129/SvJ strain is approximately fourfold greater than the spontaneous activity levels seen in Fig. 1A. To confirm this finding, a separate experiment was conducted in which locomotor activity was assessed in 129/SvJ mice that received a saline injection vs. animals placed directly into the test environment. Again, saline-injected mice had an approximately fourfold higher activity level than noninjected animals

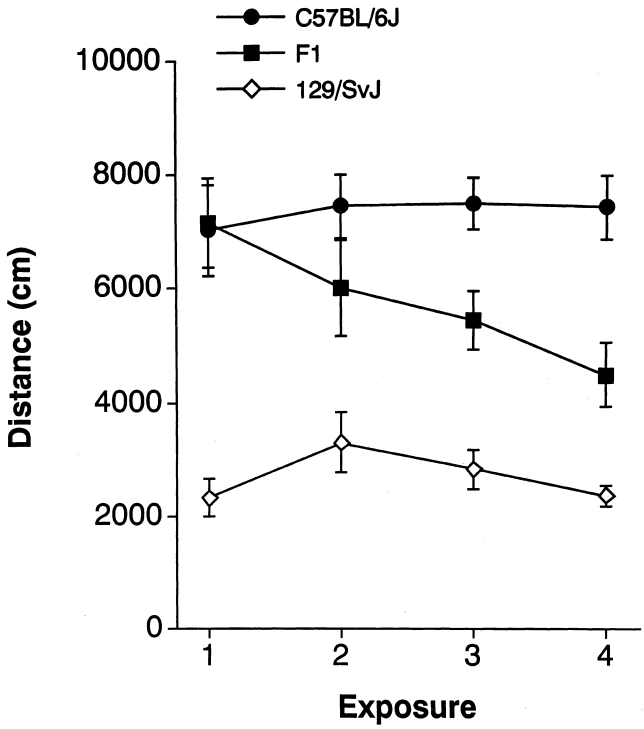

FIG. 2. Development of long-term habituation in C57BL/6J and 129/SvJ inbred mice and their F1 cross. Locomotor activity (total distance traveled) was assessed over a 2-week period on a Monday, Thursday, Monday, Thursday test schedule. Each point represents the mean ± SEM total distance traveled in a 30-min test period (n = 6 for each strain).

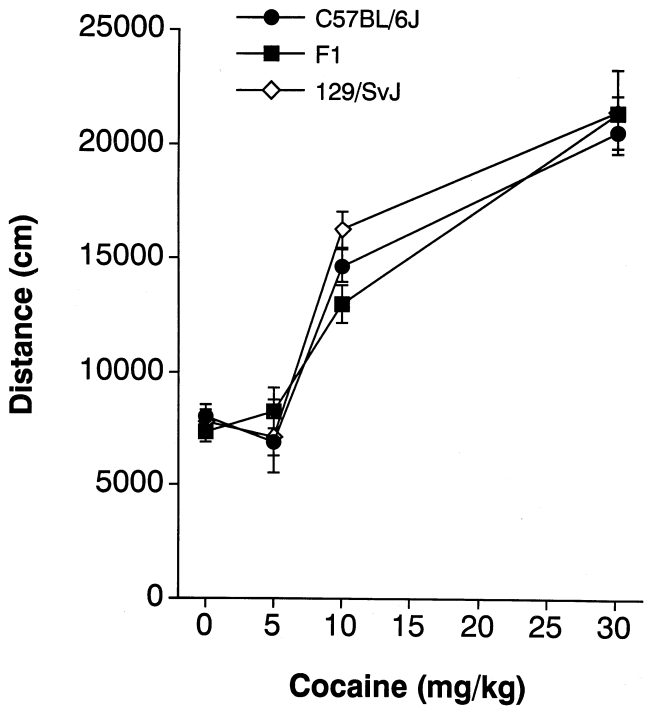

FIG. 3. Cocaine effects on locomotor activity in C57BL/6J and 129/SvJ inbred mice and their F1 cross. Cocaine was administered at the indicated doses, and locomotor activity was assessed for 30 min immediately thereafter. Each point represents mean ± SEM distance traveled for 6–10 mice.

[injected: 8088 ± 942; noninjected: 2548 ± 303; $t(10) = 5.60$, $p < 0.0001$]. Thus, the activity levels seen in the 129/SvJ mice after cocaine injection are a result of both a reaction to the injection and the stimulant effects of the drug.

Cocaine Conditioned Place Preference

Cocaine's reward and reinforcing properties were assessed using the conditioned place preference test. The initial side preferences prior to cocaine conditioning were similar among the three genotypes; percentages of time spent in the nonpreferred side (which would become the drug-paired side in the conditioning phase) were as follows: C57BL/6J, 36.2 ± 3.4%; 129/SvJ, 38.0 ± 5.0%; and F1, 42.5 ± 6.0%. Comparison of the dose–response curves for the two parental strains by two-way ANOVA resulted in significant main effects for strain [$F(1, 48) = 12.64$, $p < 0.001$] and dose [$F(3, 48) = 2.95$, $p < 0.05$], as well as a strain × dose interaction [$F(3, 48) = 2.88$, $p < 0.05$]. Cocaine conditioning resulted in a significant change in preference in favor of the drug-paired side in the C57BL/6J strain (one-way ANOVA, dose: $F(3, 28) = 13.15$, $p < 0.001$). Both the 5- and 10-mg/kg conditioning doses resulted in a significant increase as compared with the saline-conditioned group (Bonferroni–Dunn post hoc test, $p < 0.05$) (Fig. 4A). In contrast, under the same conditioning regimen, mice of the 129/SvJ strain showed no significant shift toward the cocaine-paired side at any dose [one-way ANOVA, dose: $F(3, 28) = 0.98$, $p = 0.42$]. The 5-mg/kg dose of cocaine, which gives the greatest difference between the two parental strains, was chosen to assess cocaine reward in the F1 generation. With the same conditioning regimen, the F1 generation showed a shift toward the drug-paired side similar in magnitude of that seen for the C57BL/6J strain [one-way ANOVA, genotype: $F(2, 17) = 12.64$, $p < 0.001$] (Fig. 4B). This pattern of results is indicative of preference for a more intense drug response.

DISCUSSION

As this and previous studies have so thoroughly demonstrated, the genetic makeup of an animal can greatly influence a variety of behaviors, including response to drugs of abuse (5,6,12,17,18). The present study characterizes cocaine response in a previously uncharacterized mouse strain, 129/SvJ, as well as in the widely used C57BL/6J strain and in F1 ani-

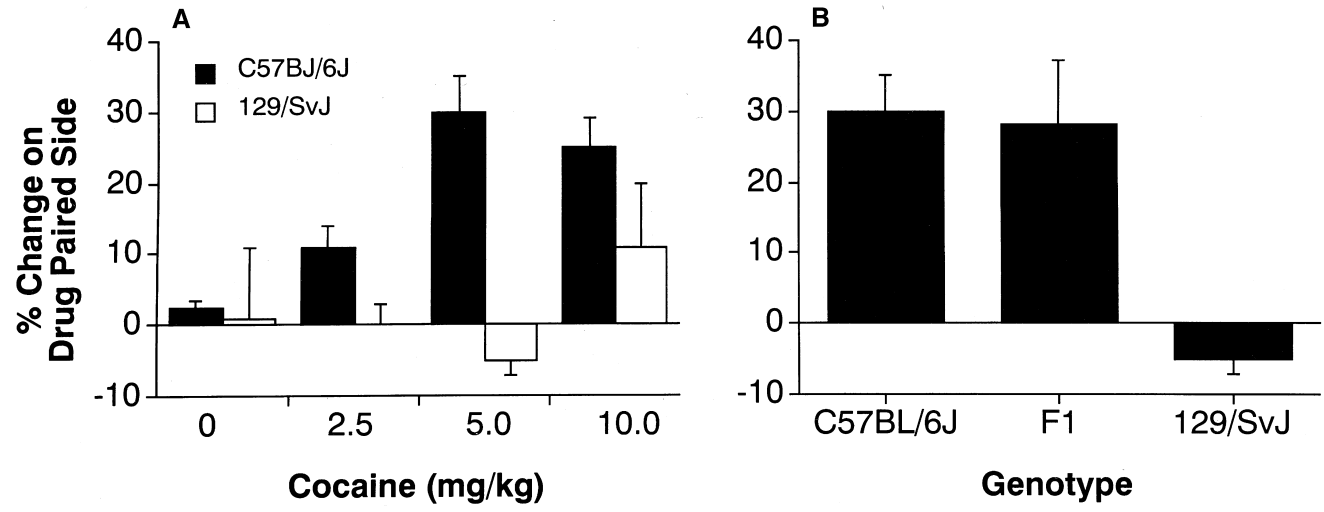

FIG. 4. (A) Cocaine conditioned place preference in C57BL/6J and 129/SvJ inbred mice. Mice were conditioned at the indicated doses as described in the Methods section. Each bar represents the mean change ± SEM of % time spent on the cocaine-paired side for 6–8 animals per dose. (B) Cocaine conditioned place preference in C57BL/6J, 129/SvJ, and F1 mice. Mice were conditioned with 5 mg/kg of cocaine as described. Each bar represents the mean change ± SEM of % time spent on the drug-paired side for 6–8 mice.

mals derived from them. This characterization and future studies focusing on other pharmacological agents will lay the foundation for a better understanding of the range of responses to be expected when examining newly developed "knockout" strains.

In addition to characterizing the two progenitor strains, this study has demonstrated that it is important for investigators to consider that genes do not always act in a purely additive fashion. Therefore, knowing the phenotypes of the parental strains does not necessarily define the full range of responses in animals derived from their crossing. This is an important and often overlooked consideration, given that many of the recently developed "knockout" mice are a null mutation on a polygenic background. The potential of dominance and epistatic interaction exists when mating two strains, which can result in phenotypes unlike either parental strain. The F1 generation is an important tool in assessing the degree of dominance, i.e., allelic interactions within a locus (8). In the present study, animals from the F1 generation are indistinguishable from the C57BL/6J parental strain in spontaneous activity levels and sensitivity to cocaine's rewarding properties. This pattern of results indicates that the alleles influencing these two phenotypes in the C57BL/6J are dominant to those in the 129/SvJ strain.

When examining phenotypes that are influenced by a number of genes, it is also possible to see results that would not be predicted from knowing the phenotypes of the parental strains. This is clearly demonstrated by the results from the long-term habituation studies. Neither of the two parental strains showed any development of habituation upon repeated exposure to the test environment. In striking contrast, however, the F1 animals did show habituation, with a significant 50% reduction in activity levels over the 2-week time span. These sorts of "unpredictable" results point to the importance of testing a large number of animals to confirm that an observed phenotype is due to genetic manipulation rather than the genetic background of the mouse.

In the present study, the 129/SvJ strain presents several interesting phenotypes. While mice of this strain have very low spontaneous locomotor activity levels, they are very sensitive to the locomotor activating effects of cocaine, which produces activity levels equivalent to those seen in the C57BL/6J strain. However, this result is confounded by the dramatic increase in activity in response to saline injection. This stress response could also be influencing the poor development of cocaine conditioned place preference: the present experiments indicate that 129/SvJ mice, under conditions that produce strong place preference in C57BL/6J mice, show no positive cocaine effect. It is possible, thus, that the stress of injection is negating the reward and reinforcing effects of cocaine such that conditioning has no effect and place preference never develops.

Finally, knowledge of the potential range of responses in a variety of inbred strains will better enable investigators to choose "ideal" genetic backgrounds for manipulation and/or backcrossing a mutation onto. For example, the C57BL/6J strain is one of the most drug preferring and accepting strains known (17,18). If the genetic manipulation proposed by investigators is hypothesized to enhance drug acceptance, the C57BL/6J strain would not be the best strain to work with, because these mice are already at the upper limits of acceptance, thus making any further increase nearly impossible.

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