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Pharmacologic and Behavioral Responses of Inbred C57BL/6J and Strain 129/SvJ Mouse Lines

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HOMANICS, G. E., J. J. QUINLAN AND L. L. FIRESTONE. *Pharmacologic and behavioral responses of inbred C57BL/6J and Strain 129/SvJ mouse lines*. PHARMACOL BIOCHEM BEHAV **63**(1) 21–26, 1999.—Gene-targeting technology is creating an explosion in the number of animals available with single gene mutations that affect the function of the central nervous system. Most gene-targeted mice are produced on a mixed genetic background of C57BL/6J and substrains of Strain 129. Understanding the behavioral characteristics and responses to various drugs of these parental strains is vital to interpreting data from gene-targeted mice. We directly compared C57BL/6J and Strain 129/SvJ mouse lines on several behavioral paradigms and in response to several hypnotic and anesthetic drugs. Compared to Strain 129/SvJ mice, C57BL/6J animals are more sensitive to the hypnotic effects of midazolam, zolpidem, and propofol, less sensitive to etomidate and ethanol, and do not differ in sensitivity to Ro15-4513 or pentobarbital. These strains do not differ in their sensitivity to the motor ataxic effects of the volatile anesthetics enflurane or halothane. However, Strain 129/SvJs are more sensitive to the immobilizing effects of halothane but not enflurane. Motor coordination differs initially, but with repeated testing strain differences are no longer apparent. Strain 129/SvJ mice are more anxious on the elevated plus maze and open-field activity assays. Thus, these mouse strains harbor polymorphisms that influence some, but not all, traits of interest to behavioral neuroscientists. © 1999 Elsevier Science Inc.

Ethanol Anesthetics Anxiety Locomotor activity Inbred mice

RECENT advances in genetic technology have made it possible to create genetically engineered mice that harbor very specific alterations in single genes of interest. These genetically engineered mice are providing sensational insights into a diverse array of biologic processes as well as providing extremely useful animal models of human disease states. Recently, considerable attention in the mouse research community has focused on the effects of the genetic background on which the targeted gene modifications are maintained (2,5,7,9, 15,22).

To create genetically engineered mice [see (20)] that harbor specific mutations in single genes, investigators typically conduct the gene targeting in mouse embryonic stem cells that are derived from various substrains of Strain 129 mice. This mouse strain is used because it has been the only strain identified that readily yields robust embryonic stem cell lines that maintain pluripotency even after extended in vitro culture. Correctly targeted embryonic stem cells are microinjected into C57BL/6J blastocysts to create chimeric mice. Transmission of the genetically altered embryonic stem cell genome through the germline by mating chimeras to C57BL/6J mice results in animals that are heterozygous for the targeted allele. Interbreeding of such heterozygous mice is used to produce mice that are wild type, heterozygous, and homozygous for the targeted allele. The genetic background of these mice is thus a random mixture of C57BL/6J and Strain 129.

It is on this mixed background that analysis of most gene targeted mouse lines is conducted. For many experiments this is without serious consequence. However, for some experiments this mixed genetic background can be problematic (2,5,7,9,15,22). A mixed background often increases animal-to-animal variability. This may make small changes due to the

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altered gene difficult to detect. Perhaps a greater concern is that if the two parental strains carry polymorphic alleles for genes that influence the trait of interest, segregation of these alleles (which may or may not be linked to the targeted allele) may confound interpretation of the effect of the genetically altered allele.

Because many gene targeting studies are focused on behavioral characteristics including response to various drugs, it is important to understand the response of the commonly used background inbred mouse strains. While the C57BL/6J strain has been extensively characterized, Strain 129/SvJ has received little attention, and furthermore, these two strains have not been directly compared. We, therefore, conducted a direct comparison of C57BL/6J and Strain 129/SvJ mice on three behavioral test paradigms (open-field activity, accelerating rotarod, and elevated plus-maze) and on response to numerous sedative/hypnotic drugs including ethanol and anesthetics.

METHOD

All experiments were approved by the Institutional Animal Care and Use Committee. C57BL/6J and Strain 129/SvJ male mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and acclimated to the animal facility for at least 1 week before being used for experiments. All mice were 8–14 weeks of age at the time of analysis; within each experiment all mice were of similar age. Mice were group housed (two to four per cage) under a 12-h light/dark cycle (lights on at 0700 h) and provided ad lib access to food and water. All experiments were conducted in an isolated behavioral testing room in the animal facility to avoid external distractions. Unless otherwise noted, experiments were performed under the ambient temperature and lighting conditions of the test room. Three groups of mice (10–20 mice of each strain per group) were sequentially subjected to the following tests as described below. Group I: midazolam sleep time, pentabarbital sleep time, halothane tail clamp, ethanol ±Ro15-4513 sleep time, halothane loss of righting reflex, and rotarod. Group II: halothane loss of righting reflex, rotarod, halothane tail clamp, midazolam sleep time, pentabarbital sleep time, zolpidem sleep time, ethanol ±Ro15-4513 sleep time, and open-field assay. Group III: enflurane tail clamp, open-field assay, elevated plus-maze, etomidate sleep time, propofol sleep time, ethanol ±Ro15-4513 sleep time, and enflurane loss or righting reflex. All mice were allowed to recover for at least 1 week between each drug treatment. For each experiment, approximately equal numbers of C57BL/6J and Strain 129/SvJ mice were used during each test session.

Sensitivity to midazolam (Versed, Hoffman-LaRoche Inc., Nutley, NJ; 45.0 mg/kg, IP), zolpidem (Sigma Chemical Co., St. Louis, MO; 60.0 mg/kg, IP), pentobarbital (Abbot Labs, Chicago, IL; 31.0 mg/kg, IP), etomidate (Amidate, Abbot Labs, Chicago, IL; 20.0 mg/kg, IP), and propofol (Diprivan, Zeneca Inc., Wilmington, DE; 30.0 mg/kg, IV) was determined using the standard sleep time assay (17). Undiluted clinical grade midazolam stock solution (5.0 mg/ml) was injected at 0.009 ml/g body weight. Zolpidem was dissolved in 0.9% saline to 6.0 mg/ml and injected at 0.01 ml/g body weight. Pentobarbital was diluted from clinical stock solution in 0.9% saline to 3.1 mg/ml and injected at 0.01 ml/g body weight. Etomidate, undiluted from clinical grade stock solution (2.0 mg/ml) was injected at 0.01 ml/g body weight. Propofol was diluted from clinical stock solution in 0.9% saline to 6.0 mg/ml and injected into the tail vein at 0.005 ml/g body weight. All sleep time experiments were performed between 0900 and 1300 h. Briefly, sleep time assays were conducted as follows. Mice were injected with drug and when ataxic placed in the supine position in V-shaped plastic troughs until they were able to right themselves three times within 30 s. Sleep time was defined as the time from being placed in the supine position until regaining of the righting reflex. Effect of strain was compared by Student's *t*-test. During all sleep time assays, normothermia was maintained with a heat lamp. Mice that failed to lose the righting reflex (misplaced injections) or had a sleep time greater than two standard deviations from the group mean were excluded from the analysis unless otherwise noted.

Sleep time in response to ethanol (Pharmaco Products Inc., Brookfield, CT; 3.5 g/kg, IP) was determined following pretreatment with vehicle or Ro15-4513 (Research Biochemicals, Natick, MA; 10 mg/kg, IP) as described (13). Ethanol was diluted in 0.9% saline (17.5% w/v) and administered at 0.02 ml/g of body weight. Ro15-4513 was dissolved in a drop of Tween 80, diluted in saline (1.0 mg/ml), and sonicated. Ro15-4513 or vehicle (Tween 80 in saline) was administered at 0.01 ml/g of body weight 15 min prior to injection with ethanol. Effect of strain, pretreatment, and their interaction was compared by ANOVA.

Response of mice to the volatile anesthetics halothane (Ayerst, New York, NY) and enflurane (Anaquest, Madison, WI) was determined using the loss of righting reflex and tail clamp/withdrawal assays as described (13,19). All mice were tested between 1000 and 1400 h. Briefly, for the loss of righting reflex assay, mice were placed in individual wire mesh cages mounted on a carousel in a sealed Plexiglas chamber. Chamber temperature of 33–35°C and ambient CO_2 tension of <0.05 atmosphere were maintained during experiments. Anesthetics were delivered from agent-specific vaporizers and chamber concentration of anesthetics was measured on line with a Datex Capnomac II. Mice were equilibrated for 15 min at each anesthetic concentration before the carousel was rotated at 4 rpm for five complete revolutions. Mice were scored for the righting reflex in a quantal fashion: mice that passively rolled over two times were scored as positive for loss of the righting reflex. Between each test concentration, the chamber was evacuated and mice were allowed to recover in air for 15 min. Tail clamp/withdrawal assays also utilized a sealed chamber and conditions as described above. Briefly, mice were equilibrated at each anesthetic concentration for 15 min before a tail clamp stimulus (hemostat, 5 inch straight) was applied to the base of the tail, and organized motor withdrawal was scored in a quantal fashion. Mice were allowed to recover in air for 30 min before assay at the next concentration of anesthetic. Concentration–response data for loss of righting reflex and tail clamp/withdrawal assays were fit to a logistic equation (25) and half-effect concentration (EC_{50}), slopes, and estimates of the standard errors were determined. Strains were compared using the Z statistic (1).

Motor coordination was assayed using an accelerating rotarod (Basile Rota-Rod Treadmill, Model 7650, Stoelting, Wood Dale, IL). The rod is 3.0 cm in diameter with a knurled Perspex surface, and is suspended 16.0 cm above the metal timer trip plates. Briefly, mice were tested on this apparatus as follows. Mice were acclimated to the stationary rod for 3 min on the day prior to the first of 8 consecutive daily trials. During each test session, each mouse was placed on the stationary rod for ~30 s, after which the rod accelerated from 3 to 19 rpm over 180 s. The time from the start of acceleration to the time each mouse fell from the rod was automatically re-

corded. All rotarod experiments were conducted between 1000 and 1400 h. Effect of strain on the time on rod per trial was compared by Student's *t*-test.

Basal locomotor activity, anxiety, and the anxiolytic and hyperlocomotor effects of ethanol (1.5 g/kg, IP) were assessed using the elevated plus-maze (16,18). Each mouse used was naive to the test apparatus, and all experiments were conducted between 0900–1100 h. The maze was constructed exactly as described (16): the floor of the apparatus was black Plexiglas, open arms lacked rims, arms were 30 × 5 cm, and connected by a 5 × 5 cm center platform, two of the four arms were enclosed by 15 cm high clear Plexiglas walls, and the entire apparatus was mounted 38.5 cm above the floor. Mice were moved into the behavioral testing room at least 1 h prior to testing. Mice were pretreated with 0.01 ml/g body weight of ethanol (15% w/v in saline) or vehicle (0.9% saline) 30 min prior to being placed on the central platform facing an open arm. The number of entries onto open and closed arms as well as the time spent on both types of arms during a 5-min test period were recorded by an investigator in the corner of the behavioral testing room. An arm entry was defined as a mouse having entered an arm with all four legs. Total number of arm entries, an indicator of locomotor activity, was the sum of entries onto open and closed arms. Entries onto the central platform were not included in any calculations. The data collected was used to calculate percentage of time on open arms [time on open arms/(time on open arms + time on closed arms) × 100], and percentage of entries onto open arms [number open arm entries/(number open arm entries + number closed arm entries) × 100]. Before testing each mouse, the apparatus was wiped with water, 70% ethanol, and dry towels and allowed to air dry for 5 min. Effect of strain, treatment (vehicle vs. ethanol), and their interaction on total number of arm entries, percentage of entries onto open arms, and percentage of time spent on open arms were compared by ANOVA (8,18).

Emotionality and locomotor activity were analyzed using an open-field activity assay. Each mouse used was naive to the test apparatus. Two experiments were conducted. The first experiment compared C57BL/6J and Strain 129/SvJ mice at ~9 weeks of age, and the second compared these same strains at ~14 weeks of age. All experiments were conducted between 0800–1100 h. The test arena was constructed of clear Plexiglas (46 × 46 cm), and was divided into 16 squares (11.5 cm^2) by lines drawn on the floor of the apparatus. The four squares not bounded by the walls of the test arena were referred to as center squares. Mice were moved into the behavioral testing room at least 1 h prior to testing. Each mouse was placed into a corner square of the arena and allowed to freely explore for 10 min. During this period, mice were observed by an investigator in the corner of the behavioral testing room for rearing behavior, total number of squares entered, and number of center squares entered. An entry into a square was defined as having all four feet in the square at one time. At the conclusion of the test period, the number of fecal boli was counted. The apparatus was cleaned between mice as described above for the plus maze. Strain differences were compared by Student's *t*-test.

For all statistical comparisons, $p \leq 0.05$ was considered significant.

RESULTS

C57BL/6J and Strain 129/SvJ male mice were compared for hypnotic response to several sedative/hypnotic drugs using the standard sleep time assay (17). Strain 129/SvJ mice were significantly less sensitive to the effects of midazolam, zolpidem, and propofol, as evidenced by the ~38–70% shorter sleep time compared to C57BL/6J mice (Table 1). It is noteworthy that following injection with midazolam and zolpidem, nine (47% of total) and four (27% of total) Strain 129/SvJ mice, respectively, failed to lose the righting reflex (i.e., sleep time = 0 min), whereas all C57BL/6J mice exhibited a positive response (i.e., sleep time > 0 min). It was also observed that immediately upon the start of the propofol injection into the tail vein of Strain 129/SvJ mice, these mice very quickly relaxed and injections proceeded without incident. In marked contrast, upon injection of C57BL/6J mice, many continued to move and twitch, which consequently prevented injection of the complete dose of propofol. These incomplete injections were excluded from the analysis. The underlying basis for this observation may be pharmacogenetic or pharmacokinetic differences between strains or alternatively a difference in sensitivity to the venous irritation induced by propofol.

Analysis of etomidate and ethanol induced sleep time revealed that Strain 129/SvJ mice are significantly more sensitive to the hypnotic effects of these drugs compared to C57BL/6J mice (Table 1). The relative sensitivities of these two strains to ethanol are similar to that reported for the

TABLE 1

SLEEP TIME ANALYSIS IN RESPONSE TO MIDAZOLAM (45 MG/KG), ZOLPIDEM (60 MG/KG), PROPOFOL (30 MG/KG), ETOMIDATE (20 MG/KG), ETHANOL (3.5 G/KG) ± Ro15-4513 (10 MG/KG), AND PENTOBARBITAL (31 MG/KG)

	Sleep Time (min ± SEM)						
Strain	Midazolam*	Zolpidem†	Propofol‡	Etomidate§	Vehicle + ethanol	Ro15-4513 + ethanol#	Pentobarbital¶
C57BL/6J	69.2 ± 4.6 (20)	23.6 ± 2.6 (14)	9.5 ± 0.8 (7)	57.2 ± 3.6 (18)	55.7 ± 4.5 (16)	45.8 ± 2.5 (17)	25.7 ± 3.4 (10)
129/SvJ	21.2 ± 5.7 (19)	15.2 ± 3.1 (15)	4.9 ± 0.2 (19)	74.8 ± 4.2 (19)	69.8 ± 3.6 (16)	58.2 ± 4.1 (15)	32.5 ± 4.9 (10)

*$p < 0.0001$; included in the sleep time measurement for Strain 129/SvJ mice are nine animals that did not loose the righting reflex.
†$p = 0.05$; included in the sleep time measurement for Strain 129/SvJ mice are four animals that did not loose the righting reflect.
‡$p < 0.0001$.
§$p < 0.003$.
#$p < 0.006$ for effect of pretreatment; $p < 0.001$ for effect of strain; $p = 0.82$ for pretreatment by strain interaction.
¶One mouse of each strain failed to loose the righting reflex following injection; these mice were excluded from the analysis.

TABLE 2
BEHAVIORAL RESPONSES TO THE VOLATILE ANESTHETICS ENFLURANE AND HALOTHANE ON THE LOSS OF RIGHTING REFLEX AND TAIL CLAMP/WITHDRAWAL ASSAYS

		EC_{50}	
Strain (*n*)	Anessthetic	(%ATM ± SE)	Slope ± SE
Loss of righting reflex assay			
C57BL/6J (20)	enflurane	1.20 ± 0.04	20.01 ± 4.29
129/SvJ (20)	enflurane	1.26 ± 0.03	27.49 ± 5.73
C57BL/6J (24)	halothane	0.75 ± 0.03	14.88 ± 2.70
129/SvJ (24)	halothane	0.71 ± 0.03	15.37 ± 2.77
Tail-clamp/withdrawal response			
C57BL/6J (20)	enflurane	2.23 ± 0.10	12.86 ± 2.79
129/SvJ (20)	enflurane	2.05 ± 0.09	14.88 ± 2.70
C57BL/6J (22)	halothane	1.59 ± 0.07*	16.68 ± 3.25
129/SvJ (24)	halothane	1.42 ± 0.05	19.11 ± 3.61

*$p = 0.05$.

closely related C57BL/6N and Strain 129/J mouse lines (3). The "ethanol antagonist," Ro15-4513 (23) was equally effective in reducing the duration of ethanol induced sleep time in mice of both strains (Table 1). We conclude that ethanol-induced sleep time, but not its antagonism by Ro15-4513, may be influenced by genetic background when these strains are used for the creation of genetically altered mice.

Sleep time in response to pentobarbital did not differ between strains (Table 1). The values presented are similar to previously published values for F_2–F_3 C57BL/6J × Strain 129/Sv/SvJ hybrid mice (13). Thus, analysis of pentobarbital induced sleep time of genetically altered mice maintained as C57BL/6J × Strain 129/SvJ hybrids is unlikely to be confounded by the genetic background.

C57BL/6J and Strain 129/SvJ mice were examined for sensitivity to the volatile anesthetic agents halothane and enflurane using two standard assays that measure different behavioral end points of anesthesia. The loss of righting reflex assay was used to measure the motor ataxic effects of the anesthetics, and the tail clamp/withdrawal assay was used to measure the immobilizing effects of these drugs. As shown in Table 2, slope of the concentration response curves were not significantly different between strains in any of the assays, allowing valid comparisons of their EC_{50} values. These mouse strains did not differ in response to either anesthetic in the loss of righting reflex assay or to enflurane in the tail clamp/withdrawal assay. However, Strain 129/SvJ mice were more sensitive (i.e., a lower EC_{50}) than C57BL/6J mice to the immobilizing effects of halothane. Previously published results from wild-type F_2-F_4 C57BL/6J × Strain 129/Sv/SvJ hybrid mice (13,19) are similar to those values reported here for the parental inbred strains.

C57BL/6J and Strain 129/SvJ mice were also compared for motor coordination using 8 consecutive daily trials on the accelerating rotarod. As shown in Table 3, C57BL/6J mice initially outperformed Strain 129/SvJ mice during the first two trials. However, by trial 3, there was no difference between the two mouse strains. These results suggest that comparison of genetically altered mouse lines maintained on a C57BL/6J × Strain 129/SvJ hybrid background be performed during consecutive daily trials became the results of the initial trials may be confounded by the genetic background.

The elevated plus-maze was used to assess basal locomotor activity, anxiety, and the hyperlocomotor and anxiolytic effects of ethanol. C57BL/6J and Strain 129/SvJ mice did not differ in basal locomotor activity, as indicated by total arm entries (Table 4). A low dose of ethanol significantly increased locomotion equally in both strains of mice. The greater percentage of entries into open arms for C57BL/6J mice compared to Strain 129/SvJ mice indicates that C57BL/6J mice are significantly less anxious than Strain 129/SvJ mice. In both strains, ethanol exerted an anxiolytic effect indicated by the increase in the percentage of open-arm entries. However, there was a difference between strains, as indicated by the significant strain × treatment interaction. Ethanol exerted an extremely large increase in percent of open-arm entries in Strain

TABLE 3
PERFORMANCE OF C57BL/6J AND STRAIN 129/SvJ MICE ON THE ACCELERATING (3–19 RPM OVER 180 S) ROTAROD DURING EIGHT CONSECUTIVE DAILY TRIALS

Strain	*n*	Trial 1*	Trial 2†	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7	Trial 8
C57BL/6J	24	133.3 ± 12.2 (46)	152.7 ± 7.6 (43)	157.9 ± 9.7 (70)	162.2 ± 7.2 (65)	159.7 ± 6.9 (55)	174.7 ± 3.2 (85)	169.2 ± 6.9 (85)	178.1 ± 1.3 (90)
129/SvJ	24	91.1 ± 12.9 (17)	119.9 ± 10.0 (18)	134.1 ± 8.2 (19)	151.4 ± 8.3 (52)	155.5 ± 8.0 (57)	166.0 ± 5.7 (67)	172.9 ± 3.0 (62)	173.5 ± 2.9 (71)

Results (in seconds) are presented as means ± SEM.
Numbers in parentheses indicate the percentage of mice remaining on rotarod for the entire 180-s trial.
*$p < 0.03$.
†$p < 0.02$.

129/SvJ mice in contrast to a rather modest increase in C57BL/6J mice. Percentage of time spent on open arms yielded similar results to the percentage of entries onto open arms; however, only the effect of treatment was statistically significant. The results presented are consistent with previous reports of moderate levels of anxiety related behaviors (24) and anxiolytic responses to ethanol (10) for the C57BL/6J strain. However, the hyperlocomotor effects of ethanol observed for the C57BL/6J mice in the present study are at odds with a similar study demonstrating that C57BL/6J mice are resistant to this effect of ethanol (10). The reason for this discrepancy is unknown.

The standard open-field assay was used to compare these mouse strains for locomotor activity, exploratory behavior, and anxiety at two different ages. The first group of mice were tested at an age of ~9 weeks before they were subjected to extensive handling as part of other assays. Locomotor activity (indicated by total squares crossed during test period), the number of fecal boli, and thigmotaxis (proportion of time spent in outer squares of test arena) did not differ between strains (Table 5). Only rearing behavior, an indicator of anxiety (a lower incidence of rearing reflects greater anxiety) was significantly different, with Strain 129/SvJ mice displaying less rearings per test period compared to C57BL/6J mice. These results are consistent with an earlier report in which young C57BL/6J mice were compared to Strain 129/J mice (4).

A second group of mice were tested in the open-field assay at ~14 weeks of age after they had been subjected to considerable handling as part of other experiments. Strain 129/SvJ mice displayed significantly less locomotor activity compared to C57BL/6J mice on this test. Thigmotaxis did not differ between strains. Similar to the experiment conducted at ~9 weeks of age, Strain 129/SvJ mice continued to display greater levels of anxiety, as indicated by the lower number of rearings and the greater number of fecal boli compared to C57BL/6J animals. The differences observed between the animals tested at 9 and 14 weeks of age may be age related or may be due to environmental factors/stresses such as handling. Additional experiments are required to fully understand these differences. Based on the results presented, it is recommended that to minimize effects of genetic background on open-field activity of gene-targeted mouse lines, experiments be conducted on young, naive animals.

DISCUSSION

Emerging genetic technologies are making it possible to study the consequences of individual gene modifications on whole animal behavioral and pharmacologic responses. The gene targeting technology is being employed at an exponential rate to generate mice with precise mutations in individual genes of interest that affect the function of the central nervous system (21). Due to technical considerations, most gene-targeted mice are maintained and tested on a mixed genetic background of C57BL/6J and various substrains of Strain 129. Because inbred strains of mice such as these often differ on behavioral and pharmacologic responses [for review, see (6)], the phenotypic effect of the introduced mutation is often confounded by polymorphic alleles contributed by each of the parental mouse strains. Therefore, it is critically important to understand the response of the parental strains used in the construction of genetically altered animals. To this end, we have directly compared the inbred C57BL/6J and Strain 129/SvJ mouse lines for performance in several behavioral paradigms and for response to several sedative/hypnotic agents. These inbred mouse lines exhibit substantial differences on some, but not all behavioral parameters measured. Similarly, these two mouse lines also display divergent responses to some, but not all drugs tested.

In gene-targeting experiments, genetic heterogeneity between parental strains such as that presented here may make the response of hybrid offspring so variable that minor differences due to the intentionally introduced mutation are undetectable. For example, mice lacking the α_6 subunit of the γ-aminobutyric acid type A receptor are rather unremarkable for numerous behavioral and pharmacologic responses (11,13).

TABLE 4

COMPARISON OF C57BL/6J AND STRAIN 129SvJ MOUSE STRAINS ON THE ELEVATED PLUS MAZE ASSAY FOLLOWING PRETREATMENT WITH VEHICLE (SALINE) OR ETHANOL (1.5 g/kg)

Strain (*n*)	Treatment	Total Arm Entries*	%Entries Open Arms †‡§	% Time on Open Arms#
C57BL/6J (17)	vehicle	13.3 ± 1.2	23.4 ± 3.1	16.5 ± 2.8
129/SvJ (17)	vehicle	14.2 ± 1.2	5.7 ± 3.1	7.6 ± 4.4
C57BL/6J (18)	ethanol	21.1 ± 1.7	28.7 ± 4.5	23.1 ± 4.6
129/SvJ (18)	ethanol	19.1 ± 1.4	27.8 ± 5.4	30.8 ± 6.4

Results are means ± SEM.
*Effect of treatment, $p < 0.0001$.
†Effect of strain, $p < 0.04$.
‡Effect of treatment, $p < 0.002$.
§Strain by treatment interaction, $p = 0.05$.
#Effect of treatment, $p < 0.003$.

TABLE 5

COMPARISON OF C57BL/6J AND STRAIN 129/SvJ MOUSE STRAINS ON A 10-MIN OPEN-FIELD ACTIVITY ASSAY

Strain (*n*)	Total Squares	Center Squares (%of Total)	Outer Squares (% of Total)	Rearings	Fecal Boli
9 Weeks of age					
C57BL/6J (20)	253.3 ± 10.8	17.9 ± 1.0	82.1 ± 1.0	32.0 ± 2.6*	3.3 ± 0.7
Strain 129/SvJ (19)	242.6 ± 8.5	17.5 ± 1.3	82.5 ± 1.3	13.2 ± 1.8	4.6 ± 0.5
14 Weeks of age					
C57BL/6J (15)	242.1 ± 9.1*	15.5 ± 1.2	84.5 ± 1.2	20.3 ± 3.3*	2.3 ± 0.5†
Strain 129/SvJ (15)	135.3 ± 11.6	14.2 ± 2.1	85.8 ± 2.1	2.4 ± 0.7	5.3 ± 0.7

*$p < 0.0001$.
†$p < 0.001$.

A different problem exists if genetic differences between parental strains that influence the phenotype under investigation are linked to the gene targeted mutation (22). These linked genetic polymorphisms will cosegregate and influence the phenotype. The genes linked to the targeted mutation will be Strain 129 derived in knockout animals, and the genes linked to the wild-type version of the targeted gene will be C57BL/6J derived in control animals. Because of this, it will be impossible to definitively attribute the phenotype of a gene-targeted mouse strain to either the targeted gene or to the cosegregating genes if the phenotype of the gene-targeted mouse strain is similar to the phenotype of the parental Strain 129 animals. If the phenotype of the knockouts is very different from the parental Strain 129 mice, then the phenotype must be due to the targeted gene and not to the linked genes.

Genetic background does not appear to contribute to the behavioral phenotype of the recently described mouse line that lacks the β_3 subunit of the γ-aminobutyric acid type A receptor (12,19). The response of these knockouts to the various sedative/hypnotic agents tested here does not parallel the response observed in Strain 129/SvJ mice. In contrast, decreased rotarod performance observed in the dopamine D_2 receptor knockout mouse strain has recently been demonstrated to be due to Strain 129 genes that cosegregate with the targeted mutation (14).

In conclusion, these two inbred strains of mice that are commonly used as background stock for gene targeting studies (i.e., Strain 129/SvJ and C57BL/6J) differ on some, but not all, behavioral and pharmacologic end points tested. These inbred mouse strains must, therefore, harbor polymorphic alleles that influence many traits that are of interest to behavioral neuroscientists [see also (6)]. In a gene-targeted animal that is maintained and tested on a mixed genetic background of these two strains, polymorphic loci may cosegregate with the gene-targeted allele, potentially making the results difficult to interpret (2,9,15,22). Therefore, when conducting gene-targeting experiments on a mixed genetic background, it is important to exercise extreme caution in the interpretation of the results, as the phenotype of interest may be influenced by the genetic background in addition to the targeted allele. A thorough understanding of the response of the parental mouse strains is critical to the proper interpretation of the phenotype.

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