Behavioral Differences among C57BL/6 Substrains: Implications for Transgenic and Knockout Studies

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Abstract

Separate breeding colonies of C57BL/6 ("B6") mice maintained at the Jackson Laboratories ("J") and NIH ("N") have led to the emergence of two distinct substrains of C57BL/6 mice: C57BL/6J and C57BL/6N. Molecular genetic studies indicate simple sequence-length polymorphisms, single-nucleotide polymorphisms, and copy-number variants among B6 substrains that may contribute to phenotypic differences. We examined differences in motor coordination, pain sensitivity, and conditional fear in the C57BL/6J strain and three N strains: C57BL/6NCrl (Charles River), C57BL/6NTac (Taconic), and C57BL/6NHsd (Harlan Sprague Dawley). Male C57BL/6J mice demonstrated enhanced motor coordination, as measured by the rotarod assay, markedly enhanced pain sensitivity in two assays of acute thermal nociception (e.g., tail withdrawal and hot plate), and a reduced level of conditional fear. The tail withdrawal result was confirmed in a separate laboratory. We also provide a table reviewing previously reported behavioral differences among various B6 substrains and discuss the significance of environmental differences due to obtaining mice form different vendors. These data may be seen as a potential problem and as a potential opportunity. Great care must be taken when working with mice engineered by using B6 embryonic stem cell lines because control groups, backcrosses, and intercrosses could inadvertently introduce behaviorally significant polymorphic alleles or environmental confounds. On the other hand, deliberate crosses between B6 substrains may provide an opportunity to map polymorphic loci that contribute to variability in a trait on largely homogenous backgrounds, which has the potential to improve mapping resolution and aid in the selection of candidate genes.

Keywords

C57BL/6J; pain; nociception; rotarod; fear; learning

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INTRODUCTION

C57BL/6 ("B6") mice are one of the oldest and most widely used inbred strains in biomedical research. The assembly of the mouse genome used C57BL/6J mice as the reference strain (Waterston et al., 2002). More recently, multiple international mutagenesis programs have chosen to use embryonic stem (ES) cells derived from B6 mice (rather than mice of a 129-substrain origin) to create transgenic and knockout mice with an isogenic B6 background, despite technical challenges associated with ES cells derived from these strains (Collins, Rossant, & Wurst, 2007). In order to overcome these challenges, a variety of different B6 substrains are being examined to identify ES cells that are amenable for these applications.

When ES cells were first used for targeted mutagenesis by homologous recombination (e.g., knockout mice), several different 129 substrains were used as the donor strain. The precise substrain was not always documented, and it took time for investigators to appreciate that 129 substrains can differ highly on a genetic level (Simpson et al., 1997; Threadgill, Yee, Matin, Nadeau, & Magnuson, 1997) and on a phenotypic level (Cook, Bolivar, McFadyen, & Flaherty, 2002). The ambiguities in documentation of the 129 substrains and the interchangeable use of them in transgenic and knockout studies added further complexity to the well-known problems of a mixed 129 and B6 genetic background that may interact with the phenotype (Gerlai, 1996), as well as the "hitchiking donor gene" problem that persists even after several generations of backcrossing to the B6 strain (Lathe, 1996).

Similar to prior treatment of 129 substrains, there continues to be an implicit assumption that B6 substrains can be used interchangeably. However, accumulating molecular genetic studies indicate that this is a dangerous assumption. Multiple branches of the B6 lineage arose in the early 1950s and have been maintained as separate breeding colonies since that time; two branches in particular are denoted as C57BL/6J ("J" for The Jackson Laboratory) and C57BL/6N ("N" for National Institutes of Health) (Morse, 1978). Isolation and genetic drift of these colonies has resulted in the emergence of genetically distinct substrains. One study found 13 of 867 simple sequence-length polymorphism ("SSLP") markers (1.5%) were different between C57BL/6J and C57BL/6NCrl (Charles River) (Hovland, Cantor, Lee, Machado, & Collins, 2000). Another study found 12 of 342 SSLP markers (3.5%) were different between C57BL/6J mice versus C57BL/6N mice from Taconic Farms (C57BL/6NTac) (Bothe, Bolivar, Vedder, & Geistfeld, 2004). Further, a genetic analysis of six B6 embryonic stem-cell lines indicated differences in 35 of 275 SSLP markers (12.7%) between lines (Hughes et al., 2007). In addition to the possibility for random fixation of new mutations (Specht & Schoepfer, 2001), selection for residual heterozygosity has been implicated in explaining the large number of polymorphisms among B6 substrains (Petkov et al., 2004).

Multiple copy-number variants have also been discovered among B6 substrains (Egan, Sridhar, Wigler, & Hall, 2007; Mulligan et al., 2008), as well as between C57BL/6J strains obtained from different breeders (Watkins-Chow & Pavan, 2008). Additionally, expression profiling of several brain regions from C57BL/6J and C57BL/6NCrl indicate differential regulation of multiple transcripts between the two strains (Mulligan et al., 2008). C57BL/6J mice exhibit a higher recombination frequency than C57BL/6N mice when crossed with the BALB/c donor strain (Goto, Ebukuro, & Itoh, 2005), suggesting the possibility of different numbers and, possibly, types of recombinations when using these two substrains as the recipient strain for backcrossing mice containing the 129-derived donor transgene.

The purpose of this study was to further explore the degree of behavioral differences that can be expected among B6 substrains. We report differences in rotarod performance and

pain sensitivity, using two tests of acute thermal nociception (e.g., hot plate and tail withdrawal). We chose to examine one phenotype, tail withdrawal sensitivity, in two separate laboratories to determine the reliability of strain differences across different testing environments. Last, we also examined fear learning, since this has been most extensively examined among B6 substrains (Radulovic, Kammermeier, & Spiess, 1998; Siegmund, Langnaese, & Wotjak, 2005; Stiedl et al., 1999). While C57BL/6J and C57BL/6NCrl (Charles River) were used in all experiments, for purely historical reasons, either the C57BL/6NTac (Taconic) or the C57BL/6NHsd (Harlan Sprague Dawley) strain (but not both) were also included. The goal of this study was not to exhaustively test all available B6 substrains nor was it to clearly discriminate between the possible genetic or environmental differences associated with these different vendors. Rather, we have reported new examples of phenotypic differences among B6 substrains to illustrate the problem and have gone on to review the already published findings by compiling a summary table to demonstrate the extensive phenotypic differences among various B6 substrains.

METHODS

Mice

Male C57BL/6J (The Jackson Laboratory, Bar Harbor, Maine, USA), C57BL/ 6NCrl (Charles River Laboratories, Inc., Wilmington, Massachusetts, USA), C57BL/6NTac (Taconic Farms, Germantown, New York, USA), or C57BL/ 6NHsd (Harlan Sprague Dawley, Inc., Indianapolis, Indiana, USA) mice arrived on the same day for each experiment and were allowed at least 1 week to habituate to the vivarium before simultaneous testing at 9–13 weeks of age. The historical relationship among the B6 substrains used in this study are shown in Figure 1A. All mice were housed 4 per cage, provided unlimited access to food and water, maintained on a 12-hour light-dark cycle, and tested during the light phase. All experiments were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committees at the University of Chicago (Chicago, Illinois, USA) and the University of California, Los Angeles (Los Angeles, California, USA).

Tail Withdrawal and Hot Plate

The 48.0°C tail withdrawal assay was conducted at both the University of California and the University of Chicago, and the 52.5°C hot-plate assay was conducted in Los Angeles, using procedures described previously (Bryant, Eitan, Sinchak, Fanselow, & Evans, 2006). With respect to the tail-withdrawal data in Chicago, the order of testing (tail withdrawal or fear; see below) was counterbalanced and mice were allowed 2 weeks of rest between the two assays. There was no effect of testing order, nor did it interact with the strain differences (data not shown). In Los Angeles, hot-plate testing was conducted first, followed by tail withdrawal 1 hour later.

Mice were transported from the vivarium to the procedure room next door and were allowed 1 hour to habituate in their home cages. In the tail-withdrawal assay, mice were gently restrained in a cotton restraint and the latency to withdrawal the tail from a hot waterbath (48.0±0.1°C) was recorded with a stopwatch to the nearest 0.1 second. A 15-second cutoff was employed to prevent tissue damage. In the hot-plate assay, mice were placed inside a acrylic cylinder (7.5 cm diameter–13 cm height) on top of a hot plate (52.5±0.1°C) and the latency to lick the hindpaw or jump was recorded. A cut-off latency of 60 seconds was used to prevent tissue damage.

Rotarod

Mice previously tested for pain sensitivity were allowed 3 weeks of rest before being tested on the rotarod apparatus in Los Angeles (TSE Systems, Midland, Michigan, USA). Mice were placed on the rotarod while it was rotating at 5 rpm. The rod was then accelerated to 60 rpm over a 3-minute interval, and the latency to fall off of the rod was recorded. Mice were tested twice separated by a 30-minute rest period and the times were averaged. The test was repeated 1 week later.

Fear Conditioning

Fear conditioning was assessed in Chicago, using the Actimetrics FreezeFrame system (www.actimetrics.com/freezeframe) to estimate freezing, as described previously (Kim & Fanselow, 1992; Ponder et al., 2007). The procedure consisted of 3 consecutive days of training and testing. On each day of testing, mice were removed from the vivarium, transported to the behavioral testing room, and allowed to habituate in their home cages for 30 minutes. Mice were placed in the conditioning chamber and, 3 minutes later, received 2 pairings of a 30-second 3-kHz tone (CS) of 85–90 dB coterminating with a 2-second, 0.5-mA foot shock (US) separated by a 30-second intertrial interval. The mice were returned to their home cage 30 seconds after the last shock. On Day 2, animals were placed in the same chamber as on Day 1, but no tones or shocks were presented. Freezing to context was defined by the period between 30 and 180 seconds. The initial 30 seconds was excluded due to high variability in freezing behavior during this period. On Day 3, freezing to an altered context was measured (Ponder et al., 2007), followed by freezing to the tone, which was presented in the same manner as on Day 1 with the exception that no foot shocks were delivered. The altered context consisted of a different experimenter testing the mice and different olfactory, visual, tactile, and auditory stimuli (Ponder et al., 2007). Freezing to the altered context was defined by the period from 30 to 180 seconds on Day 3. Tone freezing was defined by the average of the freezing response to each tone presentation (180–210 and 240–270 seconds), also on Day 3. All freezing data are presented as a percentage of the indicated time interval.

Statistical Analysis

Analysis of variance (ANOVA), followed by Fisher's PLSD test, was used to analyze the pain, rotarod, and fear data (e.g., strain as the between-subjects factor).

RESULTS

Rotarod

The results for the rotarod assay are presented in Figure 1B. There was no interaction of strain with trial either within a session or between sessions. Thus, the four trials from the two sessions were averaged and one-way ANOVA indicated a main effect of strain ($F_{2,21} = 24.168$; $P<0.05$). C57BL/6J mice demonstrated significantly longer latencies than either C57BL/6NCrl mice or C57BL/6NTac mice, and C57BL/6NCrl mice showed significantly longer latencies than C57BL/6NTac mice ($P<0.05$) (Figure 1B).

Tail Withdrawal and Hot Plate

The results of the tail withdrawal assay are shown in Figure 1C and 1D. In comparing tail-withdrawal sensitivity between C57BL/6J, C57BL/6NCrl, and C57BL/6NTac strains (Los Angeles), there was a main effect of strain ($F_{2,39} =12.75$; $P<0.05$). C57BL/6J mice demonstrated significantly shorter latencies than either C57BL/6NCrl mice or C57BL/6NTac mice ($P<0.05$). Further, C57BL/6NTac mice demonstrated significantly shorter latencies than C57BL/6NCrl mice ($P<0.05$) (Figure 1c).

We replicated the strain difference between C57BL/6J and C57BL/6NCrl in tail-withdrawal sensitivity in a separate laboratory (Chicago) and tested a different third N strain, C57BL/6NHsd (Figure 1D). One-way ANOVA indicated a main effect of strain ($F_{2,46}$ =13.78; P <0.05). C57BL/6J mice demonstrated significantly shorter latencies than either C57BL/6NCrl mice or C57BL/6NHsd mice (P<0.05) (Figure 1D).

The results of the hot-plate assay are shown in Figure 1E. There was a main effect of strain ($F_{2,39}$ =7.66; P<0.05). C57BL/6J mice demonstrated significantly shorter latencies than C57BL/6NCrl mice and C57BL/6NTac mice (P<0.05) (Figure 1E).

Conditional Fear

The results for conditional fear for C57BL/6J, C57BL/6NCrl, and C57BL/6NHsd strains are shown in Figure 1f–h. There were no significant effects of strain on pretraining freezing to the context ($F_{2,46}$=2.15; P<0.05: C57BL/6J= 5.42±1.36; C57BL/6NCrl=7.68±1.57; C57BL/6NHsd=4.016±0.79). Thus, conditional contextual fear is represented as% freezing on test day. In examining conditional contextual fear, one-way ANOVA indicated a significant effect of strain ($F_{2,46}$ =12.39; P<0.05). C57BL/6NCrl mice exhibited significantly more freezing than C57BL/6J and C57BL/6NHsd mice (P<0.05) (Figure 1F).

In the altered context, there was a main effect of strain ($F_{2,46}$ =6.32; P< 0.05). C57BL/6NCrl mice froze more than C57BL/6J mice (P<0.05) (Figure 1G). For all three strains, the amount of freezing to context was significantly greater than pretraining freezing or freezing to the altered context (P<0.05; data not shown).

The results for conditional tone fear are presented in Figure 1H. There was no significant effect of initial freezing to the tone on Day 1 ($F_{2,46}$ =1.39, P<0.26; C57BL/6J=12.86±2.51; C57BL/6NCrl=8.65±1.44; C57BL/6NHsd=12.29±1.69). Thus, conditional tone fear is represented as% freezing on test day. One-way ANOVA indicated that there was no significant effect of initial freezing to the tone ($F_{2,46}$ =1.15, P<0.32) (Figure 1H).

We examined postshock freezing during training as an indirect measurement of sensitivity of the unconditional stimulus (i.e., shock). There was no effect of strain on freezing following the first ($F_{2,46}$ <1.75, P<0.18; C57BL/6J=10.57±3.13; C57BL/6NCrl=17.26±3.58; C57BL/6NHsd=9.94± 2.39) or second shock ($F_{2,46}$ =3.06, P>0.05; C57BL/6J=18.64±4.27; C57BL/6NCrl=23.98±3.74; C57BL/6NHsd=12.29±1.69).

DISCUSSION

This study clearly demonstrates significant behavioral differences among C57BL/6J and C57BL/6N strains. These differences were often robust and could easily be misattributed to a targeted mutant allele in cases where B6 substrains were used interchangeably. With regard to motor coordination and pain sensitivity, we also observed differences within C57BL/6N substrains obtained from different vendors (Figure 1B and 1C). Behavioral differences have previously been reported between C57BL/6J substrains, but to our knowledge, this is the first report to demonstrate differences between C57BL/6N substrains. Table 1 provides a list of studies explicitly reporting B6 substrain differences in behavioral phenotypes. Our findings extend this list to include rotarod performance and pain sensitivity.

We observed large differences among B6 substrains in rotarod performance, with the C57BL/6J strain demonstrating approximately a two-fold greater latency to fall off than the C57BL/6NTac strain (Figure 1B). This is in contrast to a previous finding demonstrating no difference between C57BL/6J and C57BL/6NTac (Bothe et al., 2004), which, as noted by the researchers, could be influenced by any number of rotarod parameters (e.g., rod

diameter, rod material, acceleration rate, or number of trials) (Rustay, Wahlsten, & Crabbe, 2003). However, in the present study, the number of trials did not influence the strain differences in rotarod performance, as there was no interaction of strain with rotarod latency either within session (two trials) or between the two sessions (data not shown). Although the source of discrepancy is not clear, it is obvious that there are rotarod conditions in which B6 substrains can differ dramatically in performance, and thus, this should be considered when choosing the most appropriate background strain for transgenic and knockout experiments.

The C57BL/6J strain was most sensitive in both the tail-withdrawal and hot-plate tests (Figure 1C–E), indicating a generalized enhanced sensitivity to acute thermal pain in this substrain. In addition, we observed a low level of context fear in C57BL/6J mice (Figure 1F and 1H), which is generally consistent with previous observations (Radulovic et al., 1998; Siegmund et al., 2005; Stiedl et al., 1999). Importantly, because the laboratory environment can affect mouse strain differences in behavioral phenotypes, and because the stability of these differences across labs is extremely behavior dependent (Crabbe, Wahlsten, & Dudek, 1999), we confirmed the strain difference in one of the novel phenotypes, tail withdrawal sensitivity, in a separate laboratory. The marked sensitivity of C57BL/6J mice to thermal pain confirms that they are a poor choice of background strain for testing this phenotype in genetically modified mice (Mogil and Wilson, 1997), and that the DBA/2J strain may be a more suitable control strain due to its moderate sensitivity across multiple assays (Lariviere, Chesler, & Mogil, 2001).

Strain differences in pain sensitivity to the unconditioned stimulus (e.g., foot shock) during fear conditioning might be expected to explain differences in fear learning. However, in comparing thermal pain versus conditional fear, we observed opposite results: greater pain sensitivity in C57BL/6J mice but lower levels of conditional fear. However, it is important to note that thermal pain sensitivity and shock sensitivity may be under different genetic control (Mogil et al., 1999). As an indirect measure of shock sensitivity, we measured postshock freezing following the first and second shock, and there were no strain differences (see Results). This suggests that strain differences in conditional fear are due to fear learning, rather than sensitivity to the unconditional stimulus.

We attempted to control environmental influences as closely as possible upon receiving the mice from the respective vendors. Nonetheless, numerous environmental factors prior to the arrival of the mice could contribute to the observed behavioral differences (e.g., differences in breeding and rearing conditions, handling, or embryonic environment). For example, vendor differences in animal handling by the vendors could induce different levels of chronic stress, which might account for some or all of the observed strain differences in pain (Clausing, Mothes, Opitz, & Kormann, 1997; Pieretti, d'Amore, & Loizzo, 1991) and/or conditional fear (Beane, Cole, Spencer, & Rudy, 2002; Madruga, Xavier, Achaval, Sanvitto, & Lucion, 2006; Meerlo, Horvath, Nagy, Bohus, & Koolhaas, 1999). While we examined the effects of different laboratory environment on the tail-withdrawal test, we did not breed mice from the different vendors at our vivarium, which would have allowed us to better standardize environmental conditions.

A previous study found that breeding mice in-house versus using mice shipped from a vendor does not have a major impact on behavior, provided that sufficient adaptation time is allowed after arrival (Crabbe et al., 1999). Similarly, a recent study indicated that breeding in-house versus purchasing from a vendor did not affect B6 substrain differences in alcohol preference or consumption (Mulligan et al., 2008). Both of these examples support the hypothesis that genetic differences account for the strain differences; however, our data cannot specifically address this issue. Therefore, the current differences, and many of the differences shown in Table 1, should be considered to be of unknown origin. Clearly,

anyone contemplating studies where it is necessary to use multiple B6 substrains in a single experiment should breed both strains in the same facility and should then evaluate the relevant phenotypes to determine whether genetic differences exist in the absence of obvious environmental differences.

CONCLUSION

Our observations stress the critical importance of correctly recording and reporting the source of B6 ES cells and background substrains used for the production of genetically engineered mice. Further, when crossing lines (e.g., when using Cre-Lox systems), differences in B6 substrain background could lead to unintended genetic heterogeneity, which will introduce potentially confounding genetic and phenotypic differences. Similarly, comparing homozygous knockout mice that have been produced in one substrain to wild-type mice from a different substrain or mice that were raised in a different environment could lead to erroneous conclusions.

These observations also present an opportunity. Crossing strains that are largely isogenic increases the chance that phenotypic differences are due to one or a few alleles. Thus, a cross between genetically similar B6 substrains that show a phenotypic difference would allow for mapping of the causative allele against a largely isogenic background. This approach, in combination with utilizing transcriptome differences between B6 substrains (Grottick et al., 2005; Mulligan et al., 2008), may greatly facilitate identifying candidate genes that contribute to behavioral differences. In summary, our observations reveal both the challenges and opportunities inherent in mouse genetics.

Acknowledgments

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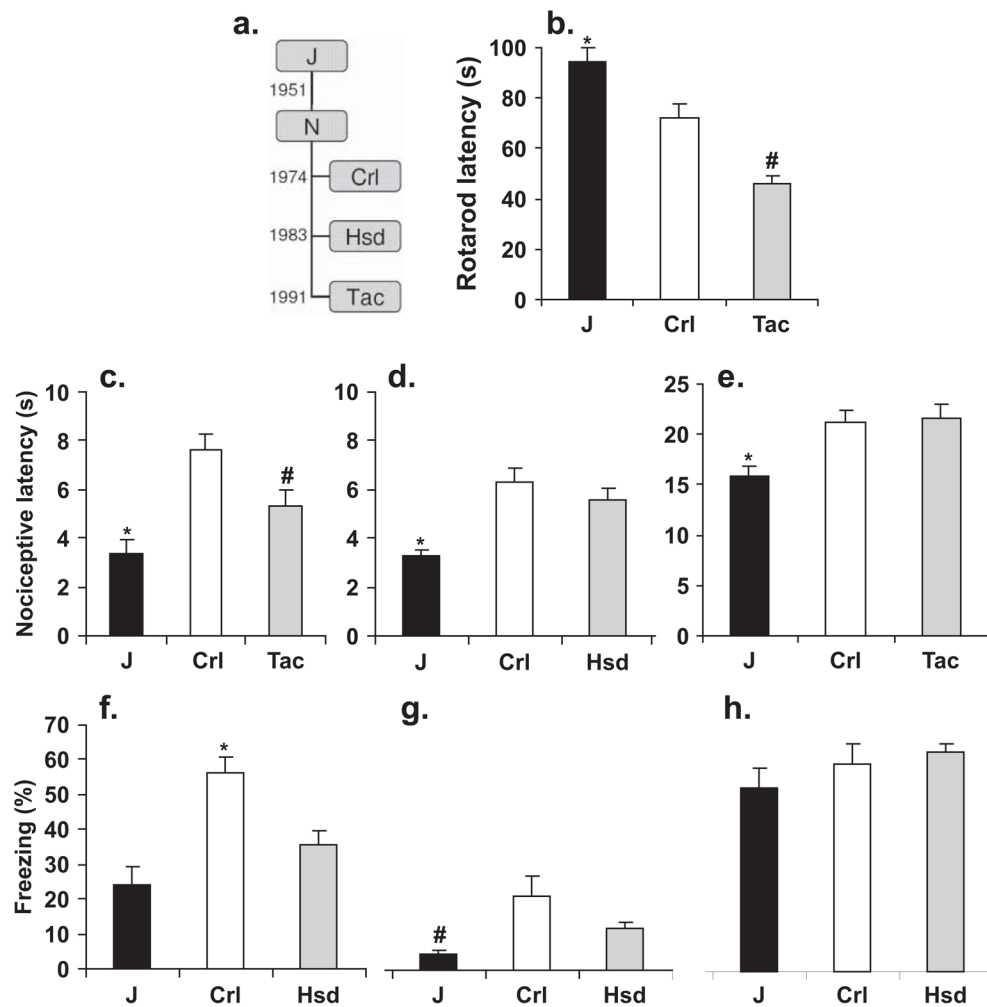
Figure 1.
(**A**) Origin and dates of separation of the B6 substrains used in this study. J=The Jackson Laboratory. N=National Institutes of Health. Crl=Charles River Laboratories. Hsd=Harlan Sprague Dawley. Tac=Taconic Farms. (**B**) Rotarod latencies (*N*=8). (**C**) Tail-withdrawal latencies (Los Angeles; *N*=14). (**D**) Tail-withdrawal latencies (Chicago; *N*=16=17). (**E**) Hot-plate latencies (*N*=14). (**F**) Context fear (*N*=16=17). (**G**) Fear in the altered context. (**H**). Tone fear. "*"= significantly different from the other two strains. "#"=significantly different from Crl. *P*<0.05 was considered significant.

Table 1

Substrain Differences in Various Behavioral Phenotypes

Study	Strains	Phenotypes showing a significant difference	Rank order
(Poley, 1972)	C57BL/6J, C57BL/6A	Alcohol preference	C57BL/6J>C57BL/6A
(Moisset, 1977)	C57BL/6J C57BL/6ByJ	d-amphetamine rearing	C57BL/6ByJ>C57BL/6J
		d-amphetamine locomotor activity	C57BL/6J>C57BL/6ByJ
(Blum, Briggs, DeLallo, Elston, & Ochoa, 1982)	C57BL/6J C57BL/6N	Alcohol preference	C57BL/6J>C57BL/6N
(Crusio, Schwegler, & van Abeelen, 1991)	C57BL/6JNmg C57BL/6JKun	Rearing	C57BL/6JKun>C57BL/6JNmg
		Wall leaning	C57BL/6JNmg>C57BL/6JKun
		Activity	C57BL/6JNmg>C57BL/6JKun
		Wood sniffing	C57BL/6JNmg>C57BL/6JKun
(Jamot, Bertholet, & Crusio, 1994)	C57BL/6JNmg C57BL/6JKun	Radial arm maze errors	C57BL/6JNmg>C57BL/6JKun
		Radial arm maze correct choices	C57BL/6JKun>C57BL/6JNmg
(Henricks, Miner, & Marley, 1997)	C57BL/6J C57BL/6ByJ	Cocaine locomotor activity	C57BL/6ByJ>C57BL/6J
		Spontaneous locomotion in a novel environment	C57BL/6ByJ>C57BL/6J
		Susceptibility to cocaine seizures	C57BL/6J>C57BL/6ByJ
(Radulovic et al., 1998)	C57BL/6J C57BL/6NCrl	Context fear	C57BL/6NCrl>C57BL/6J
(Sluyter, Marican, & Crusio, 1999)	C57BL/6JNmg C57BL/6JKun	Aggression	C57BL/6JNmg>C57BL/6JKun
		Nest building	C57BL/6JNmg>C57BL/6JKun
(Stiedl et al., 1999)	C57BL/6JOlaHsd C57BL/6NCrl	Context fear	C57BL/6NCrl>C57BL/ 6JOlaHsd
		Extinction of context fear	C57BL/6JOlaHsd>C57BL/ 6NCrl
		Extinction of tone fear	C57BL/6JOlaHsd>C57BL/ 6NCrl
(van Gaalen & Steckler, 2000)	C57BL/6J C57BL/6ChR	Digging	C57BL/6J>C57BL/6ChR
		Activity	C57BL/6J>C57BL/6ChR
(Mayorga & Lucki, 2001)	C57BL/6J C57BL/6NHsd	Tail climbing	C57BL/6J>C57BL/6NHsd
(Siegmund et al., 2005)	C57BL/6NCrl / C57BL/6JOlaHsd C57BL/6JCrl	Context fear	C57BL/6NCrl>C57BL/ 6JOlaHsd, C57BL/6JCrl
(Grottick et al., 2005)	C57BL/6J C57BL/6NHsd	Prepulse inhibition	C57BL/6NHsd>C57BL/6J
		Startle response amplitude	C57BL/6J>C57BL/6NHsd
(Khisti, Wolstenholme, Shelton, & Miles, 2006)	C57BL/6J C57BL/6NCrl	Alcohol deprivation effect	C57BL/6NCrl>C57BL/6J
		Alcohol consumption	C57BL/6J>C57BL/6NCrl
(Ramachandra et al., 2007)	C57BL/6J, C57BL/6NCrl	Alcohol preference and consumption	C57BL/6J>C57BL/6NCrl

Study	Strains	Phenotypes showing a significant difference	Rank order
(Siegmund & Wotjak, 2007)	C57BL/6JOlaHsd C57BL/6NCrl	Fear extinction	C57BL/6NCrl>C57BL/6JOlaHsd>C57BL/6JCrl
		Horizontal locomotion	C57BL/6NCrl>C57BL/6JOlaHsd>C57BL/6JCrl
		Rearing	C57BL/6JCrl>C57BL/6NCrl>C57BL/6JOlaHsd
		Light/dark box: time on dark side	C57BL/6JOla>C57BL/6NCrl>C57BL/6JCrl
		Context fear	C57BL/6NCrl>C57BL/6JOlaHsd
		Sensitized fear	C57BL/6NCrl>C57BL/6JOlaHsd
(Mulligan et al., 2008)	C57BL/6J C57BL/6NCrl	Alcohol preference and consumption	C57BL/6J>C57BL/6NCrl
Present study (Los Angeles)	C57BL/6J C57BL/6NCrl C57BL/6NTac	Tail withdrawal Latency	C57BL/6NCrl>C57BL/6J>C57BL/6NTac
		Hot-plate latency	C57BL/6NCrl, C57BL/6NTac>C57BL/6J
		Rotarod latency (s)	C57BL/6J>C57BL/6NCrl> C57BL/6NTac
Present study (Chicago)	C57BL/6J C57BL/6NCrl C57BL/6NHsd	Tail-withdrawal latency	C57BL/6NCrl, C57BL/6NHsd>C57BL/6J
		Context fear	C57BL/6NCrl>C57BL/6NHsd, C57BL/6J
		Altered context	C57BL/6NCrl>C57BL/6J

In chronological order, behavioral phenotypes are listed that have been reported to be statistically significant among various B6 substrains. The substrain nomenclature is based on those used by the authors of the indicated studies.