



ORIGINAL RESEARCH ARTICLE

A genetic screen for novel behavioral mutations in mice

DM Sayah*, AH Khan*, TL Gasperoni and DJ Smith

Department of Molecular and Medical Pharmacology, UCLA School of Medicine, 23–120 CHS, Box 951735, Los Angeles, CA 90095–1735, USA

A genetic screen using mice was performed to identify dominant loci affecting behavior. Mice were mutagenized with ENU, then bred to examine their G1 offspring for behavioral abnormalities. Potentially mutant G1 pups were screened through a variety of behavioral assays, including tests of learning and memory, sensorimotor gating, fear and anxiety, nociception (pain perception) and locomotor activity. Mice falling outside the normal performance distribution in these tests were considered potential behavioral mutants and were bred for further analysis. Outliers included both animals with very discrete defects and animals with abnormal performance across a range of tests. To date, we have identified two confirmed mutants affecting sensorimotor gating. These results provide further impetus for the use of random mutagenesis screens as a tool for dissecting the genetic basis of brain and behavior. *Molecular Psychiatry* (2000) 5, 369–377.

Keywords: genetics; behavior; animal; schizophrenia; mutagenesis; exploratory behavior; startle reaction; pain threshold; motor activity; memory

Introduction

Genetic analysis of human behavioral traits has been hindered by a number of factors, including polygenic inheritance, locus heterogeneity, and gene–environment interactions. One approach to circumventing these complexities is to use chemical mutagenesis of model organisms to screen for monogenic loci affecting behavior. This method has been successfully employed with invertebrate model systems such as *C. elegans* and *D. melanogaster* and has allowed identification of single genes influencing behavioral characteristics.^{1–5} However, there are obvious neuroanatomical differences between invertebrates and mammals. In addition, invertebrates do not display the full range of complex and varied behaviors typical of mammals, and few would argue that the spectrum of phenotypes associated with human psychiatric disorders can be effectively modeled in an invertebrate system. Apparently similar invertebrate and mammalian behavior may be under very different genetic controls and even molecularly related mutations may have contrasting phenotypes. While genes isolated through invertebrate screens often have mammalian homologues, it will be difficult, if not impossible, to directly infer from this information the alleles responsible for complex behavioral traits in mammals.

Until recently, most work on mammalian behavioral

genetics has employed targeted mutations of known genes in mice.^{6–8} While these experiments have done much to illuminate the genetic foundations underlying some behavioral traits, they are limited by the difficulty of predicting which genes or alleles will affect a particular behavior. One of the principal advantages of a classical forward genetic screen is the power to precisely select behavioral phenotypes of interest. In addition, knockout alleles may be homozygous inviable. This necessitates construction of more intricate alleles such as tissue specific knockouts before conclusions can be reached about the role of a particular gene in behavior.⁹

Genetic screens employing N-Ethyl-N-Nitrosourea (ENU) to mutagenize the mouse genome have already been used to identify *Clock*, a gene involved in circadian rhythm control.¹⁰ ENU is a powerful mutagen, inducing mainly point mutations but also causing some chromosome rearrangements. Mutation rates of up to 1 in 700 loci per gamete can be achieved with ENU treatment of male mice.¹¹ Here, we describe the strategies we have employed in a genetic screen designed to identify genetic loci affecting a variety of complex behaviors, many of which are relevant to human psychiatric illness.

In our screen, potentially mutant mice generated using ENU were tested for dominant or semi-dominant mutations affecting behavior. Recessive screens, while possible using these techniques, are more cumbersome, requiring three generations of breeding as opposed to the one generation required in a dominant screen.¹² Strains of mice carrying pre-existing chromosomal test deletions would simplify a recessive screen, but limit the study to a small area of the genome. Large-scale knockout experiments using gene trap techniques may

Correspondence: Dr DJ Smith, Department of Molecular and Medical Pharmacology, UCLA School of Medicine, 23–120 CHS, Box 951735, Los Angeles, CA 90095–1735, USA. E-mail: DSmith@mednet.ucla.edu

*These authors contributed equally to the manuscript
Received 7 July 1999; revised 4 November 1999 and 4 February 2000; accepted 4 February 2000

be the best approach to study recessive mutations, especially when work has barely begun in saturating the possible dominant mutations.¹³

Potentially mutant animals were subjected to a variety of tests. Beginning at the age of weaning (3 weeks) pups were visually inspected for any obvious morphological (runting, deformities, pigmentation) or behavioral (hyper/hypoactivity) abnormalities. Formal behavioral testing occurred at 8–10 weeks of age.

Several factors were considered in selecting tasks appropriate for the behavioral battery. First, in random mutagenesis screens, large numbers of animals must be tested to achieve full saturation of the mouse genome and identify mutations of potential interest. This imposes constraints on the number and types of behaviors that can be examined in a timely, cost-efficient manner. While more comprehensive batteries have been developed for targeted gene mutation studies,^{14,15} their applicability to large-scale mutagenesis is limited by the logistical demands of subjecting hundreds of mice to detailed behavioral and neurological examination. Furthermore, these constraints need to be reconciled with our goal of identifying mutations that will provide insights into the genetic basis of human behavioral and psychiatric illness. Consequently, we have adopted a multi-stage approach that both satisfies our need for high throughout phenotypic testing and maximizes our ability to detect novel behavioral mutants. After gross morphological examination, ENU-mutated animals are initially screened on a small battery comprised of well-documented behavioral assays. Individuals that deviate from the normal performance distribution are bred with isogenic B6 mice to confirm genetic transmission and a Mendelian dominant pattern of inheritance. If the trait displays evidence of monogenic transmission, mutant progeny are then subjected to detailed behavioral and neurological testing to more fully characterize the defect. Mice of interest are eventually bred to a permissive background strain (other than wild-type) in order to conduct genetic linkage analysis and isolate the responsible gene.

Behavioral domains chosen for investigation were sensorimotor gating, fear and anxiety in the open field, context-dependent memory, pain perception, and spontaneous locomotor activity. Sensorimotor gating is an attentional mechanism in which the brain filters out irrelevant stimuli in favor of more important sensory information.^{16,17} This behavior was assessed using prepulse inhibition (PPI) of the acoustic startle response, a well-characterized behavioral phenomenon observed in mammals. In this phenomenon, a low intensity audio prepulse inhibits the startle response elicited by a high intensity audio pulse. Schizophrenia patients show reduced prepulse inhibition of the acoustic startle response, as do animal subjects under the influence of phencyclidine (PCP), the best known pharmacological mimic of schizophrenia.^{18,19} The disrupted sensorimotor gating observed in schizophrenia patients may contribute to the profound thought disturbances associated with the disorder.^{20,21} Thus, mutations affecting this phenotype may help illuminate genes or

pathways that confer increased susceptibility to psychosis.

The open field test is also a well-established behavioral assay that can be used to quantitate a number of interesting behaviors.²² Here, it served as a simple, automatable test of fear and anxiety (or emotionality) and also exploratory behavior under stressful conditions. Emotionality is potentially relevant to a broad range of behavioral and psychiatric conditions.

Unlike most tests of learning and memory, context-dependent fear conditioning requires little training time and has been shown to be both hippocampus and amygdala dependent.^{23,24} Notably, medial temporal lobe abnormalities and learning and memory deficits have figured prominently in neuroanatomical and behavioral investigations of schizophrenia.^{25–30}

The tail flick test provides a simple and rapid measure of pain perception and sensitivity.^{31–33} It also serves as a useful complement to the fear conditioning assay, which relies on the animal's ability to remember the context of an electric shock.

Finally, spontaneous locomotor activity was included as a measure of motor function in a non-stressful environment. Motor abnormalities have been well-documented in schizophrenia,^{34,35} as well as in animals administered putative psychotomimetic agents such as amphetamine and phencyclidine.^{36,37}

Materials and methods

Mutation and mating

Male mice (C57BL/6J, 6–8 weeks old) were injected with ENU (150 mg kg⁻¹ IP). Approximately 4 weeks later, these mice entered a period of sterility that lasted until at least 10 weeks post-injection. Mice involved in productive matings prior to 10 weeks post injection were presumed to have received inadequate ENU treatment and therefore were not used to generate mutants for subsequent analysis. After the sterility period, the mutagenized males were crossed with untreated female mice (C57BL/6J, 8–10 weeks old). Pups from these matings were screened using the following assays in order: PPI, tail flick, open field, spontaneous locomotor activity, and context-dependent fear conditioning.

Behavioral testing

Behavioral tests were conducted on animals between 8 and 10 weeks of age. Animals were housed in a 12 h: 12 h light/dark cycle, with the light phase from 6 am to 6 pm. Tests were conducted between 10 am and 4 pm. All testing equipment was cleaned with 1:32 bleach solution between animals.

Prepulse inhibition To measure sensorimotor gating, the prepulse inhibition assay was employed.³⁸ This test was performed using the Startle Reflex Lab controlled by SR-Lab software (San Diego Instruments, San Diego, CA, USA). During the test, the animal was confined to a cylindrical holder situated inside a sound-attenuating chamber. Background noise was set at 65 dB. Five

types of trials were used. Pulse alone trials (P) consisted of a single white noise burst (120 dB, 40 ms). The prepulse + pulse trials (PP74P, PP77P, PP80P) consisting of a prepulse of white noise (20 ms at 74 dB, 77 dB, or 80 dB respectively) followed 100 ms after prepulse onset by a white noise pulse (120 dB, 40 ms). No-stimulus (NS) trials consisted of background noise only. Data in pulse and prepulse trials were recorded from the onset of the 120 dB pulse for 65 ms in 1-ms increments. Sessions were structured as follows: (1) 5-min acclimation at background noise level; (2) five P trials; (3) ten blocks each containing all five trials (P, PP74P, PP77P, PP80P, NS) in pseudorandom order; (4) five P trials. Inter-trial intervals were pseudorandomly distributed between 10 and 20 s. The maximum force intensity for each trial (V_{max}) was recorded as startle level. The average percent reduction in startle intensity between pulse and prepulse + pulse trials at all three prepulse levels was defined as the PPI level.

Tail flick To measure pain sensitivity, the tail flick assay was performed using a Columbus Instruments Tail Flick Apparatus. The mouse being tested was loosely restrained in a paper towel by hand. The tail of the animal rested freely in a groove in the apparatus above a shuttered lamp. A foot-trigger opened the shutter and started a timer. The heat from the lamp provided a nociceptive stimulus eventually causing the mouse to flick its tail away from the groove. The tail flick latency provided a measure of the animal's perception of pain. The lamp intensity was set at 8 out of 25 and the instrument's auto-detection capabilities were used to determine the subject's tail-flick latency. Reported tail flick values are the average of three trials.

Open field Fear and anxiety were measured using the open field test. The testing arena consisted of a circular, 1-m diameter, pressed-wood floor, enclosed by 40-cm walls. The interior of the arena was painted uniformly white and lights were mounted directly above the maze. The large, brightly-lit arena is considered an anxiety provoking-environment. Animals were tracked using the Poly-Track computerized video tracking system controlled by Chromo-Track software (San Diego Instruments). The 'central area' of the arena was defined by the central circle with radius 0.5 m, and the 'peripheral area' as the area outside the central area. Data recording began with the mouse placed in the center of the arena and continued for 5 min. Two measures of anxiety were taken: latency to leave the center of the arena and time spent in the center vs the periphery. An anxious mouse will immediately scurry to the periphery, thus registering a low latency to leave the center, and will spend nearly all of its time in the peripheral area. In contrast, a bold mouse will explore more freely. The total distance traveled by the subject was recorded as a measure of locomotor activity under stress.

Fear conditioning To assess learning and memory, animals were tested using context-dependent fear con-

ditioning. The test measures a subject's ability to remember the context in which it previously received a mild foot shock.³⁹ The fear conditioning chamber consisted of one side of a Gemini Avoidance System (San Diego Instruments). Access to the other side of the apparatus was blocked. Conditioning trials were structured as follows: the animal was placed in the chamber and after 2 min a tone (2900 Hz at 8.5 dB) was activated for 30 s. The last 2 s of the tone were paired with a foot shock (70 mA), and the animal was allowed an additional 30 s in the chamber before being removed to its home cage. In the testing phase 24 h later, the animal was returned to the conditioning chamber. Freezing behavior (defined as absence of all but respiratory movements) was assessed every 5 s for 3 min by an observer who entered data directly into a computer spreadsheet program in real time. The percentage of time the animal spent frozen was taken as a measure of fear and thus the ability to remember the previous day's conditioning trial.

Spontaneous locomotor activity Locomotor activity was measured in the dark using the Cage Rack System (San Diego Instruments) with a uniformly spaced 8 × 4 photobeam grid. The cages were 47 cm × 26 cm × 15 cm and the mice provided with food and water. Locomotor activity was measured by counting the total number of beam breaks during the 1-h testing period.

Statistical analysis

Data analysis was conducted as follows. First, for all G1 behavioral data, gender differences were assessed through one-way analysis of variance (ANOVA) and Pearson correlation coefficients were calculated to assess relationships between measures. Potentially mutant phenotypes were subsequently identified through construction of outlier (or box-and-whisker) plots (Figures 1–7). Individuals with phenotypic values exceeding 1.5 × the interquartile range were considered outliers and candidate behavioral mutants. A percentile approach is advantageous in this context because it yields a valid measure of spread for both normal and skewed distributions and it is relatively impervious to the effects of outliers.

A G2 generation was derived from outliers of the behavioral assays to wild-type (B6) mice. The G2 offspring were compared to the parental G1 population using Student's *t*-test, and the Shapiro Wilk *W* test was used to assess whether the performance distributions were non-normal. In addition, for offspring of mouse No. 113, we performed a cluster analysis of the PPI data using K-means with a grouping of two. This provided a determination of the mean and spread of the presumed mutant and wild-type offspring. In all cases, a probability level of $P < 0.05$ was used to define statistical significance.

Results

Over 400 mice have been screened on the prepulse inhibition and startle response measures and between

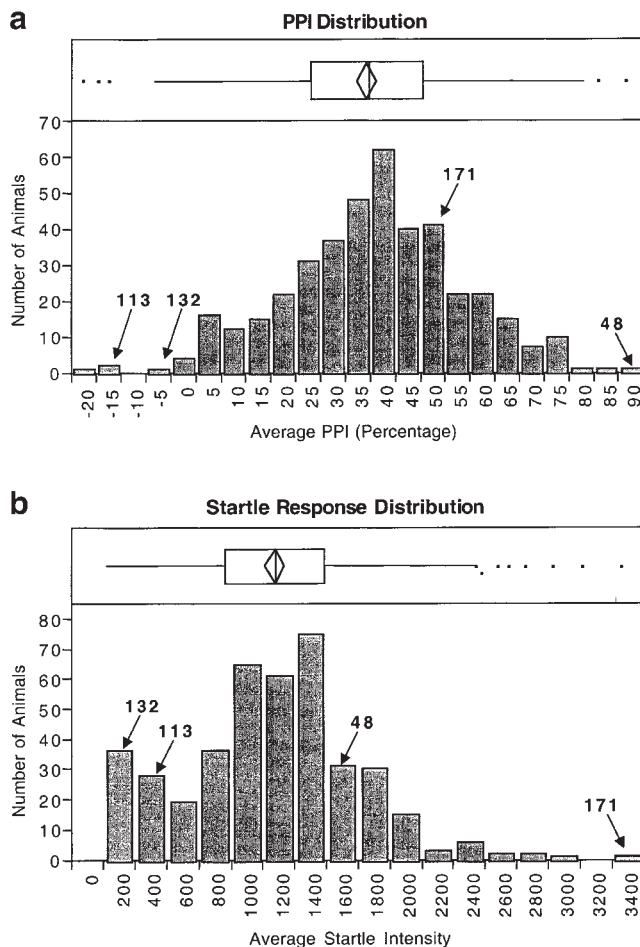


Figure 1 PPI and startle response results. (a) Distribution of PPI values ($n = 411$, mean = 35.77, SD = 17.8) averaged over all three prepulse intensity levels. PPI is expressed as percent reduction in startle from average startle response. An outlier box plot is shown above the frequency histogram. The ends of the box are the 25th and 75th quantiles, or quartiles. The diamond within the boxplot represents the 95% confidence interval of the mean. The line in the middle of the box denotes the median. Animals falling outside $1.5 \times$ the interquartile range are indicated as outliers. (b) Distribution of average startle levels ($n = 411$, mean = 1048.18, SD = 554.95). Average startle is defined as the average V_{\max} of ten pulse-alone trials.

300 and 400 on the other assays. Results from all behavioral tests were analyzed with regards to gender and no significant differences were found, except in PPI ($F[1,409] = 3.93$, $P = 0.05$) and startle response ($F[1,409] = 6.05$, $P = 0.01$). Males tended to have higher startle response levels (1109.03 ± 36.77 (mean \pm SEM) for males, 974.58 ± 40.44 for females), and slightly lower PPI (34.19 ± 1.18 for males, 37.68 ± 1.3 for females). However, the difference between means was insignificant relative to the differences seen in outliers. Consequently, for presentation all of the data have been collapsed across gender. None of the conclusions in this paper are altered when the data analysis incorporates gender considerations.

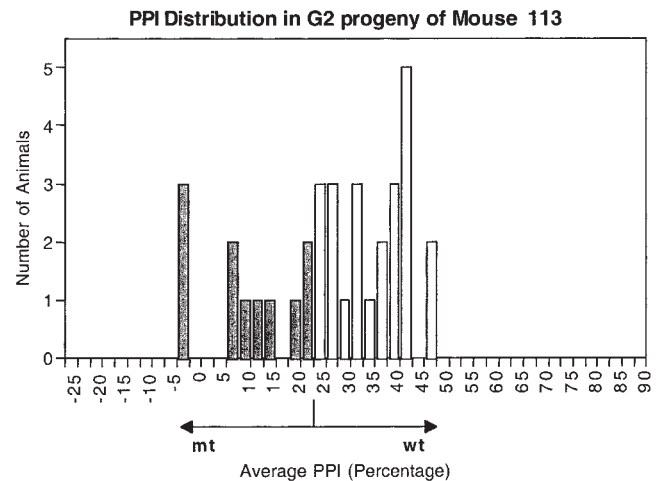


Figure 2 PPI results from G2 progeny of mouse 113 ($n = 34$, mean = 26.21, SD = 14.54). mt = mutant, wt = wild-type. The presumed 11 mutant offspring with abnormally low PPI and the presumed 23 wild-type offspring with normal PPI, as judged by K-means cluster analysis, are indicated by the arrows below the x-axis. The mutant bars in the histogram are shaded more darkly than the wild-type.

Prepulse inhibition

We have identified a number of animals that exhibited abnormal PPI at all three prepulse intensities upon both initial and repeated testing. Other candidate PPI mutants appeared normal upon retesting and such animals were not considered for further study. Similarly, animals with abnormal PPI at only one prepulse intensity were rejected. Animals with abnormal PPI included mice with both unusually high and unusually low PPI values (Figure 1). Examples include Nos 48, 113, and 132. Some mice with low PPI exhibited abnormally low startle responses (Figure 1a and b), for example No. 132, suggesting a possible hearing or other general deficit. Other mice with abnormally high or low PPI exhibited normal startle levels (Figure 1a and b, eg Nos 48 and 113), an indication of a true sensorimotor gating defect.

We have also identified animals with abnormal startle responses and normal PPI. Examples include No. 171 (Figure 1a and b). For the screened population as a whole, there was no significant correlation between PPI levels and startle magnitude ($r = -0.01319$, $P = 0.79$ when averaged over all three prepulse intensities). However, PPI at all three prepulse intensity levels was highly correlated (PP74P vs PP77P: $r = 0.7965$, $P < 0.0001$; PP74P vs PP80P: $r = 0.8235$, $P < 0.0001$; PP77P vs PP80P: $r = 0.8453$, $P < 0.0001$).

Open field

Potential mutants have been identified in several measures of the open field test. Under these stressful conditions, mice have been identified with abnormally low path lengths (Figure 4a, No. 196), abnormally low or high central dwell times (Figure 4b, Nos 196 and 400), and abnormally high latencies to leave the center (Figure 4c, No. 48). Unlike the PPI test, the open field

PPI and Startle Response in Normal and Mutant Progeny of Mouse 113

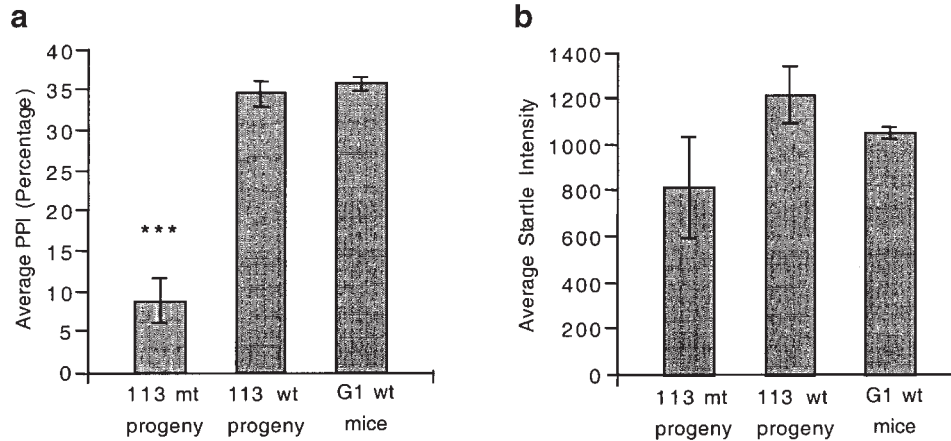


Figure 3 PPI and startle response results from presumed normal and mutant progeny of mouse 113 (mean \pm SEM). mt = mutant, wt = wild-type. (a) The PPI of the presumed mutant offspring was highly significantly less than their wild-type siblings, and also the wild-type G1 distribution ($***P < 0.0001$ comparing mutant offspring with both their wild-type siblings and the parental G1 distribution). (b) There were no significant differences in startle response.

test shows rapid habituation and cannot be effectively repeated. Therefore, all potential mutants have been bred to allow further analysis.

Context-dependent fear conditioning

A number of animals from the upper and lower ends of the fear conditioning performance distribution have been bred for further analysis (Figure 5, Nos 114 and 246). Due to the 100% and 0% boundaries inherent in this test, true outliers are difficult to identify. Therefore, animals at the extremes of the distribution are considered potential mutants. Tail flick results (Figure 6) suggest that outliers in fear conditioning are not due to abnormal pain sensitivity. Progeny of these potential fear conditioning mutants will undergo more comprehensive testing including cue-dependent fear conditioning. This test is dependent on amygdala function, in contrast to context-dependent fear conditioning which is both hippocampus and amygdala dependent.²⁴ G2 progeny of candidate mutants will also be examined using the Morris water maze task.⁴⁰

Tail flick

Some animals (Figure 6, No. 158) exhibited a tail flick latency significantly higher than the distribution of other animals. However, upon retesting, these animals' tail flick latencies were well within the normal range. Therefore, these mice were not considered to be potential mutants and were not analyzed further.

Spontaneous locomotor activity

A number of animals from both ends of the locomotor activity distribution have been bred for further analysis. These include mice with both high (Figure 7, No. 228) and low (Figure 7, No. 196) locomotor activity. Interestingly, No. 196 also displayed an abnormally

low path length in the open field assay (Figure 4). However, in general mice at the extremes of the open field path distribution do not show abnormal spontaneous locomotor activity. In fact, there is no significant correlation between path length in the open field test and spontaneous locomotor activity as measured under non-stressful conditions ($r = 0.092$). This suggests that while there is some degree of overlap, locomotor activity in the open field and spontaneous locomotor activity represent two independent indices of behavior.

G2 offspring of potentially mutant mice

We have bred potentially mutant animals to wild-type mice to confirm that the traits are transmissible and segregate as dominant loci. Although a variety of animals with deviant phenotypic scores have been bred, only two mutants, both with abnormal PPI, have been confirmed by transmission of the phenotype and these are discussed further below. Outliers in other tests have been bred, but so far there have been no other examples of genetic transmission. Outliers tested to date have been in the open field (four mice), startle response (one mouse), context-dependent fear conditioning (one mouse), and spontaneous locomotor activity (two mice).

Mouse No. 113 had abnormally low PPI but a normal startle response (Figure 1), and it would therefore be expected that 50% of its offspring will have the mutant phenotype of low PPI, and 50% would be normal. Mouse No. 113, a male, was mated to wild-type females and 34 G2 offspring were assayed using PPI (Figures 2 and 3). The mean PPI of the 34 G2 offspring was $26.21\% \pm 2.49$ which is significantly lower than the parental G1 animals ($P = 0.0024$). This is a very conservative statistical approach as the mean PPI of the 34 offspring presumably reflects a population consisting

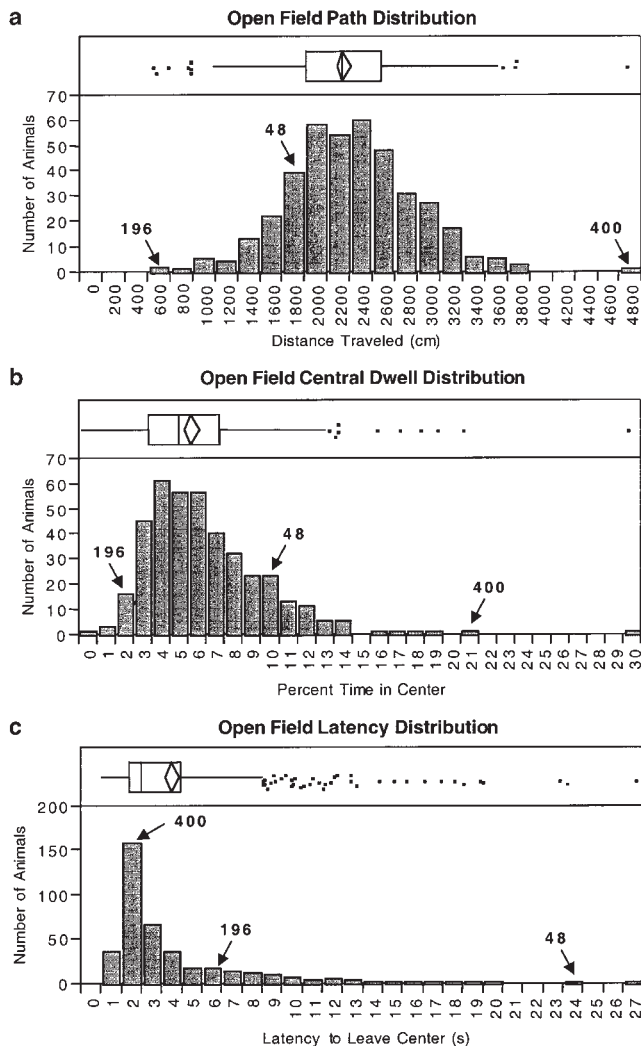


Figure 4 Open field results. (a) Path length distribution ($n = 396$, mean = 2214.96, SD = 564.36). An outlier box plot is shown above the frequency histogram. The ends of the box are the 25th and 75th quantiles, or quartiles. The diamond within the boxplot represents the 95% confidence interval of the mean. The line in the middle of the box denotes the median. Animals falling outside $1.5 \times$ the interquartile range are indicated as outliers. (b) Distribution of percent dwell time in central 50% of open field arena ($n = 396$, mean = 5.87%, SD = 3.39%). (c) Distribution of latency to leave central 50% of open field arena ($n = 396$, mean = 3.53, SD = 3.77).

of a roughly 50:50 admixture of mutant and wild-type mice. The Shapiro–Wilk W test showed that the G2 distribution was significantly non-normal ($P = 0.032$), consistent with the result expected for a bimodal distribution of PPI consisting of both mutant and wild-type mice. Cluster analysis using K-means with a group of two, corresponding to the two expected classes of offspring (mutant and wild-type) (Figure 2), revealed there were 11 mutant offspring with a PPI of $8.76\% \pm 2.77$, which is highly significantly less than the PPI of the parental G1 distribution ($P < 0.0001$) (Figure 3). There were 23 wild-type offspring with a

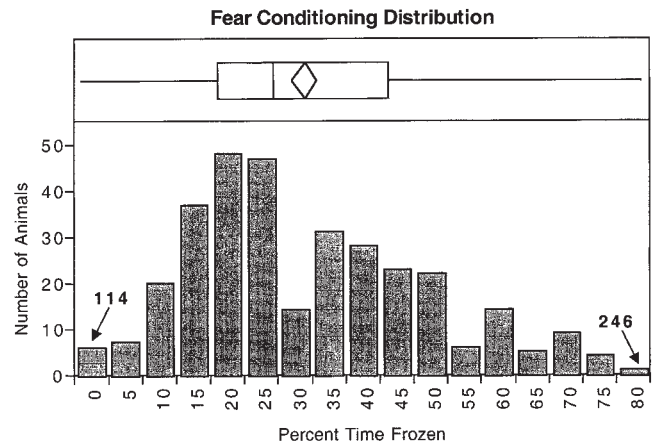


Figure 5 Fear conditioning results. Distribution of percentage of testing time spent frozen ($n = 322$, mean = 29.53, SD = 17.32). An outlier box plot is shown above the frequency histogram. The ends of the box are the 25th and 75th quantiles, or quartiles. The diamond within the boxplot represents the 95% confidence interval of the mean. The line in the middle of the box denotes the median. Animals falling outside $1.5 \times$ the interquartile range are indicated as outliers.

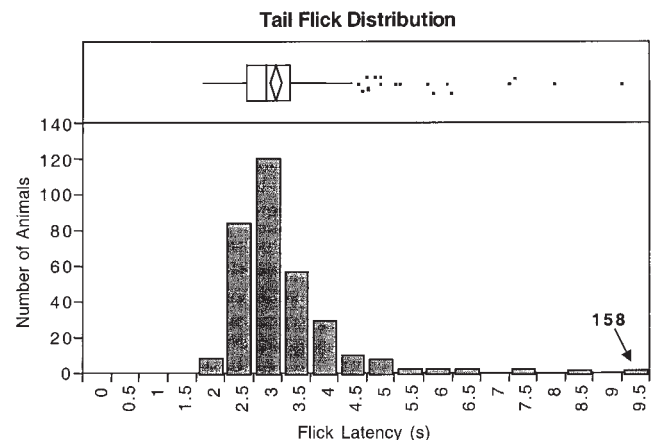


Figure 6 Tail flick results. Distribution of latency to move tail away from heat source ($n = 325$, mean = 2.98, SD = 0.91). An outlier box plot is shown above the frequency histogram. The ends of the box are the 25th and 75th quantiles, or quartiles. The diamond within the boxplot represents the 95% confidence interval of the mean. The line in the middle of the box denotes the median. Animals falling outside $1.5 \times$ the interquartile range are indicated as outliers.

PPI of $34.56\% \pm 3.63$, not significantly different from the G1 parental distribution ($P = 0.75$). The wild-type and mutant offspring were also highly significantly different from one another ($P < 0.0001$).

The new mutant is very unlikely to be simply defective in hearing or general reactivity, since the response of the mutant animals to the pulse alone, without the prepulse, is indistinguishable from the wild-type G1 distribution of startle responses ($P = 0.17$) (Figure 3). This was also true for the startle response of the presumed wild-type siblings ($P = 0.16$), as well as when all 34 offspring were pooled for comparison with the parental G1 distribution ($P = 0.71$).

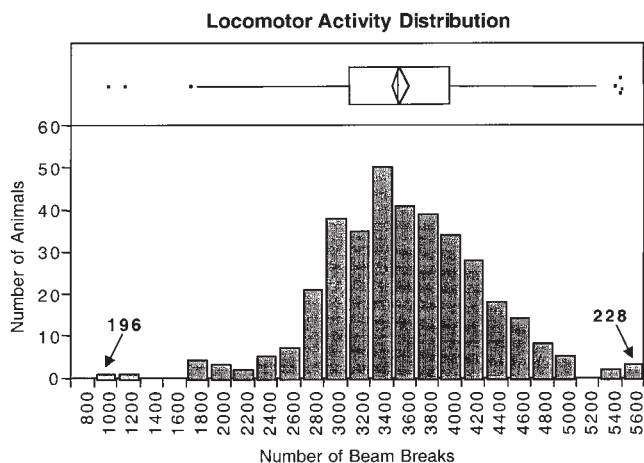


Figure 7 Spontaneous locomotor activity results. Distribution of locomotor activity as measured by the number of infrared photobeam interruptions during the testing period ($n = 359$, mean = 3487.28, SD = 684.48). An outlier box plot is shown above the frequency histogram. The ends of the box are the 25th and 75th quantiles, or quartiles. The diamond within the boxplot represents the 95% confidence interval of the mean. The line in the middle of the box denotes the median. Animals falling outside $1.5 \times$ the interquartile range are indicated as outliers.

Another potentially mutant mouse with low PPI (No. 69, not shown), was also bred with B6 females and 10 G2 offspring were examined for defective PPI. The mean of the G2 progeny ($23.32\% \pm 4.31$) was significantly different from the G1 parental population ($P = 0.03$), suggesting another dominant mutation with low PPI.

Discussion

The factors to consider when designing a genetic screen for behavioral mutants are quite different from those involved in creating a detailed phenotypic profile of an individual mutant or characterizing the effects of a drug. First and foremost, the tests in a behavioral screen must be rapid and, preferably, highly automated to facilitate screening the thousands of animals needed to achieve saturation of the genome. Thus, some well-established tests, such as the Morris water maze task,⁴⁰ which involve many days of subject training, cannot be included. A second factor to consider is test reproducibility. The ideal test is one that produces a narrow performance distribution and in which a given animal's performance is highly replicable. In addition, for optimal throughput, each test should be independent and measure unique behavioral indices. Finally, it is important that exposure to one test should not affect subject performance on another. Unfortunately, it is virtually impossible to design tests that meet all of these guidelines and still provide novel and interesting information.

As described in the above results two seemingly related behaviors, locomotor activity in the open field and spontaneous locomotor activity, are not correlated.

In contrast, mouse No. 48 is an outlier both in PPI and latency to leave the center of the open field, two ostensibly unrelated behaviors. This suggests that apparently related behaviors may be under very different genetic controls, and that diverse behaviors may in fact have a common genetic basis. These findings augur well for obtaining fundamental insights into the neural basis of behavior from genetic screens in mice.

PPI testing is based on the startle reflex and can be repeated numerous times on the same subject with consistent results. In addition, the test requires only 20 min per subject and is highly automated, making PPI an almost ideal screening test. The open field is a rapid and highly automated test, lending itself well to use in a genetic screen. Unfortunately, experiments in our lab have shown that open field behavior is not reproducible upon repeated testing of the same subject. Presumably, this effect is due to the rapid acclimation to the testing arena, making it a less anxiety-provoking environment. Spontaneous locomotor activity is an excellent screening test. Data collection is entirely automated and the test is only minimally disruptive to the subject. In addition, this test serves as a control for the open field test, distinguishing between general abnormalities in activity and alterations apparent in a stressful environment. Fear conditioning, while a fairly rapid test, requires 2 consecutive days of testing and the full attention of the researcher throughout both phases. The assay is not effectively repeatable because lingering effects of previous conditioning trials confound subsequent tests. Also, the stress associated with the footshock may have lasting effects on a subject's behavior. Evidence collected in our laboratory suggests that previous exposure to fear conditioning alters the subsequent behavior of mice in the open field. Because of possible interference with other tests, fear conditioning is the last test performed in our behavioral screen. The tail flick test, while involving extensive handling of the animals to position them properly, is nevertheless extremely rapid and reproducible, thus lending itself very well to use as a screening tool. In addition, this test is a good complement to the fear conditioning test as a control for possible decreased pain sensitivity.

The approach taken in designing our screen has been to cast a large 'net' in order to identify the maximum number of mutants. Once an interesting mutant has been identified and bred, more detailed behavioral and phenotypic analyses can be used to build a complete profile of the effects of the mutation. Although we have bred a variety of potential behavioral mutants, only the PPI assay has so far shown evidence of genetic transmission and has yielded G2 animals with an abnormal phenotype. This is not entirely unexpected, as point mutations with observable behavioral effects are likely a relatively rare occurrence and recovery of such mutants will rely on an element of chance. However, at this time we cannot exclude the possibility that the other behavioral assays are simply less sensitive to genetic perturbations and thus may not be suitable for genetic screens, although this seems an unlikely possi-

bility. Further testing of G2 animals should help to clarify this issue.

As discussed above, we have demonstrated transmission to a G2 population for two mutations affecting PPI. Mouse No. 113 appears especially promising and further genetic and behavioral characterization is ongoing. Interestingly, the broad distribution of PPI in the wild type and mutant classes of the G2 offspring of mouse 113 may suggest that this mutation has variable expressivity. This is likely a reflection of the fact that PPI is a quantitative rather than a qualitative trait, and the locus might best be regarded as a single gene contribution to a quantitative trait, which in outbred populations would have polygenic contributions. Alternatively, the non-Mendelian inheritance pattern may reflect reduced transmissibility of mutagenized chromosomes or semi-lethality of the mutation itself. While decreased transmission of ENU-treated chromosome has never been demonstrated empirically, it remains a theoretical possibility and we will continue to monitor our data for such general factors affecting genetic transmission. Semi-lethality of the mutation itself is quite possible, and this should be resolved through further genetic analysis. Nonetheless, the available evidence is consistent with a monogenic mutation, and therefore the gene should be entirely feasible to map and eventually clone.

The lack of any significant differences in startle response between mutant G2 offspring of mouse 113 and the G1 population is consistent with a defect in sensorimotor gating. Although sensory and perceptual abilities appear largely intact, additional phenotypic analysis is required to rule out the possibility of a hearing deficiency or other general deficit. Additional phenotypic assays that will be valuable in the context of the 113 mutation include the auditory brainstem response (ABR), a measure of auditory sensitivity,⁴¹ and assessment of PPI through other sensory modalities.³⁸

While the potential difficulties of performing a genetic screen in mice may seem daunting, this strategy will clearly prove useful in identifying behavior-related genes. Further, as the mouse genome project progresses, the labor involved in identification of the genes responsible for interesting behavioral phenotypes is likely to decrease considerably, with significant implications for our understanding of the genetic underpinnings of psychiatric disorders.

Acknowledgements

This work was supported by the UCLA School of Medicine and the Frontiers of Science Seed Grant Program.

References

- 1 Dudai Y, Jan YN, Byers D, Quinn WG, Benzer S. Dunce, a mutant of *Drosophila* deficient in learning. *Proc Natl Acad Sci USA* 1976; **73**: 1684–1688.
- 2 Ballinger DG, Benzer S. Photophobe (Ppb), a *Drosophila* mutant with a reversed sign of phototaxis; the mutation shows an allele-specific interaction with sevenless. *Proc Natl Acad Sci USA* 1988; **85**: 3960–3964.

- 3 Bargmann CI. Genetic and cellular analysis of behavior in *C. elegans*. *Annu Rev Neurosci* 1993; **16**: 47–71.
- 4 Sayeed O, Benzer S. Behavioral genetics of thermosensation and hygrosensation in *Drosophila*. *Proc Natl Acad Sci USA* 1996; **93**: 6079–6084.
- 5 Park EC, Horvitz HR. Mutations with dominant effects on the behavior and morphology of the nematode *Caenorhabditis elegans*. *Genetics* 1986; **113**: 821–852.
- 6 Mayford M, Abel T, Kandel ER. Transgenic approaches to cognition. *Curr Opin Neurobiol* 1995; **5**: 141–148.
- 7 Tonegawa S, Li Y, Erzurumlu RS, Jhaveri S, Chen C, Goda Y *et al*. The gene knockout technology for the analysis of learning and memory, and neural development. *Prog Brain Res* 1995; **105**: 3–14.
- 8 Wehner JM, Bowers BJ, Paylor R. The use of null mutant mice to study complex learning and memory processes. *Behav Genet* 1996; **26**: 301–312.
- 9 Tsien JZ, Chen DF, Gerber D, Tom C, Mercer EH, Anderson DJ *et al*. Subregion- and cell type-restricted gene knockout in mouse brain. *Cell* 1996; **87**: 1317–1326.
- 10 Vitaterna MH, King DP, Chang AM, Kornhauser JM, Lowrey PL, McDonald JD *et al*. Mutagenesis and mapping of a mouse gene, Clock, essential for circadian behavior. *Science* 1994; **264**: 719–725.
- 11 Russell WL, Kelly EM, Hunsicker PR, Bangham JW, Maddux SC, Phipps EL. Specific-locus test shows ethylnitrosourea to be the most potent mutagen in the mouse. *Proc Natl Acad Sci USA* 1979; **76**: 5818–5819.
- 12 Nusslein-Volhard C, Wieschaus E. Mutations affecting segment number and polarity in *Drosophila*. *Nature* 1980; **287**: 795–801.
- 13 Zambrowicz BP, Friedrich GA, Buxton EC, Lilleberg SL, Person C, Sands AT. Disruption and sequence identification of 2000 genes in mouse embryonic stem cells. *Nature* 1998; **392**: 608–611.
- 14 Crawley JN, Paylor R. A proposed test battery and constellations of specific behavioral paradigms to investigate the behavioral phenotypes of transgenic and knockout mice. *Hormones and Behavior* 1997; **31**: 197–211.
- 15 Crawley JN. Behavioral phenotyping of transgenic and knockout mice: experimental design and evaluation of general health, sensory functions, motor abilities, and specific behavioral tests. *Brain Res* 1999; **835**: 18–26.
- 16 Geyer MA, Braff DL. Startle habituation and sensorimotor gating in schizophrenia and related animal models. *Schizophr Bull* 1987; **13**: 643–668.
- 17 Geyer MA, Swerdlow NR, Mansbach RS, Braff DL. Startle response models of sensorimotor gating and habituation deficits in schizophrenia. *Brain Res Bull* 1990; **25**: 485–498.
- 18 Dulawa SC, Geyer MA. Psychopharmacology of prepulse inhibition in mice. *Chin J Physiol* 1996; **39**: 139–146.
- 19 Mansbach RS, Geyer MA. Effects of phencyclidine and phencyclidine biologs on sensorimotor gating in the rat. *Neuropsychopharmacology* 1989; **2**: 299–308.
- 20 Swerdlow NR, Braff DL, Taaid N, Geyer MA. Assessing the validity of an animal model of deficient sensorimotor gating in schizophrenic patients. *Arch Gen Psychiatry* 1994; **51**: 139–154.
- 21 Braff DL, Grillon C, Geyer MA. Gating and habituation of the startle reflex in schizophrenic patients. *Arch Gen Psychiatry* 1992; **49**: 206–215.
- 22 Walsh RN, Cummins RA. The Open-Field Test: a critical review. *Psychol Bull* 1976; **83**: 482–504.
- 23 Logue SF, Paylor R, Wehner JM. Hippocampal lesions cause learning deficits in inbred mice in the Morris water maze and conditioned-fear task. *Behav Neurosci* 1997; **111**: 104–113.
- 24 Phillips RG, LeDoux JE. Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behav Neurosci* 1992; **106**: 274–285.
- 25 Arnold SE, Franz BR, Gur RC, Gur RE, Shapiro RM, Moberg PJ *et al*. Smaller neuron size in schizophrenia in hippocampal subfields that mediate cortical-hippocampal interactions. *Am J Psychiatry* 1995; **152**: 738–748.
- 26 Falkai P, Bogerts B. Cell loss in the hippocampus of schizophrenics. *Eur Arch Psychiatry Neurol Sci* 1986; **236**: 154–161.
- 27 Gray JA, Feldon J, Rawlins JN, Hemsley DR. The neuropsychology of schizophrenia. *Behav Brain Sci* 1991; **14**: 1–84.
- 28 Kovelman JA, Scheibel AB. A neurohistological correlate of schizophrenia. *Biol Psychiatry* 1984; **19**: 1601–1621.

- 29 Nelson MD, Saykin AJ, Flashman LA, Riordan HJ. Hippocampal volume reduction in schizophrenia as assessed by magnetic resonance imaging: a meta-analytic study. *Arch Gen Psychiatry* 1998; **55**: 433–440.
- 30 Saykin AJ, Gur RC, Gur RE, Mozley PD, Mozley LH, Resnick SM *et al*. Neuropsychological function in schizophrenia. Selective impairment in memory and learning. *Arch Gen Psychiatry* 1991; **48**: 618–624.
- 31 Pick CG, Cheng J, Paul D, Pasternak GW. Genetic influences in opioid analgesic sensitivity in mice. *Brain Res* 1991; **566**: 295–298.
- 32 Pick CG. Strain differences in mice antinociception: relationship between alprazolam and opioid receptor subtypes. *Eur Neuropsychopharmacol* 1996; **6**: 201–205.
- 33 Seale TW, Nael R, Singh S, Basmadjian G. Inherited, selective hypoanalgesic response to cytisine in the tail-flick test in CF-1 mice. *Neuroreport* 1998; **9**: 201–205.
- 34 Walker E, Shaye J. Familial schizophrenia. A predictor of neuromotor and attentional abnormalities in schizophrenia. *Arch Gen Psychiatry* 1982; **39**: 1153–1156.
- 35 Walker EF, Savoie T, Davis D. Neuromotor precursors of schizophrenia. *Schizophr Bull* 1994; **20**: 441–451.
- 36 Sams-Dodd F. Effects of continuous D-amphetamine and phencyclidine administration on social behaviour, stereotyped behaviour, and locomotor activity in rats. *Neuropsychopharmacology* 1998; **19**: 18–25.
- 37 Adams B, Moghaddam B. Corticolimbic dopamine neurotransmission is temporally dissociated from the cognitive and locomotor effects of phencyclidine. *J Neurosci* 1998; **18**: 5545–5554.
- 38 Paylor R, Crawley JN. Inbred strain differences in prepulse inhibition of the mouse startle response. *Psychopharmacology* 1997; **132**: 169–180.
- 39 Fanselow MS, Bolles RC. Naloxone and shock-elicited freezing in the rat. *J Comp Physiol Psychol* 1979; **93**: 736–744.
- 40 Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. *J Neurosci Meth* 1984; **11**: 47–60.
- 41 Zheng QY, Johnson KR, Erway LC. Assessment of hearing in 80 inbred strains of mice by ABR threshold analyses. *Hearing Research* 1999; **130**: 94–107.