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NIH Swiss and Black Swiss Mice Have Retinal Degeneration and Performance Deficits in Cognitive Tests

Steven J. Clapcote,[1,*] Noah L. Lazar,[2,†] Allison R. Bechard,[1] Geoffrey A. Wood,[1] and John C. Roder[3]

Swiss mice are among the most commonly used outbred strains in biomedical research. Because prior knowledge of the baseline phenotypes of mouse strains will allow informed selection of strains for particular experiments, we sought to characterize the behavior of two previously untested outbred Swiss strains—NIH Swiss and Black Swiss—in the two most widely used paradigms for evaluating the cognitive abilities of mice. Unlike the C57BL/6J and C57BL/6J-*Tyr*$^{c\text{-}2J}$ controls, animals of both outbred Swiss strains were unable to demonstrate learning in the Morris water maze and contextual fear conditioning paradigms. A polymerase chain reaction assay revealed that all of the NIH Swiss and Black Swiss mice tested were homozygous for the recessive retinal degeneration 1 mutation of the *Pde6b* gene. Histological examination of NIH Swiss and Black Swiss mouse eyes confirmed the presence of retinal degeneration, which causes visual image blindness. These findings indicate that NIH Swiss and Black Swiss mice are visually impaired and thus may be unsuitable for use in some experiments.

Swiss mice are among the most commonly used outbred strains in biomedical research (39). Their characteristics of robustness, disease resistance, and fecundity are considered advantageous by some investigators, particularly when it is desirable to use groups of animals whose levels of heterogeneity mimic those found in human populations. Among their various roles, outbred Swiss strains are used for assessing the effects of pharmacological agents (e.g., 9, 13, 27). They are also a frequent host strain for targeted mutations (the Mouse Genome Database [25] lists 78 cases), thus providing the genetic background for numerous transgenic models of human disease (e.g., 37, 41).

The phenotype of mice carrying a targeted mutation may vary depending on the genetic background because of the presence of genetic modifiers (allelic variants at loci other than the mutated gene) in the host strain genome (reviewed in 28, 30). In this way, the characteristics of the host strain may enhance or hinder the ability to detect and analyze the phenotypic effects of the mutation. For example, C57BL/6J congenic *Slc6a4* null mutants exhibit increased anxiety-like behavior and reduced exploratory locomotion in comparison with their wild-type littermates, whereas 129S6/SvEvTac congenic *Slc6a4* null mutant and wild-type littermates do not differ (22). Prior knowledge of the baseline phenotypes of the various mouse strains available would, therefore, allow informed selection of host strains for genetic manipulation and would aid the interpretation of phenotypic testing results. With this in mind, we characterized the behavior of two previously untested outbred Swiss strains—NIH Swiss and Black Swiss—in the most widely used paradigms for evaluating the cognitive abilities of mice (11): contextual fear conditioning (38) and the Morris water maze (31). In light of the favorable general-purpose characteristics of NIH Swiss and Black Swiss mice, our objective was to assess their utility for our own research on learning and memory (10, 17).

Contextual fear conditioning is a well-established model for emotional learning that tests an animal's ability to learn and remember an association between an environment and foot shock (38). Subjects are required to attend to and learn a multiplicity of contextual cues that can be visual, olfactory, tactile, or auditory in modality. The procedure takes advantage of the natural tendency of rodents to suppress any movement besides respiration and heartbeat in response to fearful stimuli (3, 4). This "freezing" behavior has proven to be a useful measure of conditioned fear in rodents under various experimental conditions, and it is used widely to evaluate contextual learning in mice (21, 29).

The Morris water maze is a spatial navigation task that requires mice to swim in a pool of opaque water until they locate a hidden (submerged) platform (31). Spatial learning in the water maze is expected to be more dependent than is contextual fear conditioning on visual ability because, to find the platform, mice must learn to use spatial information from visual cues placed around the test room (15, 33). Evidence that spatial memory has formed after repeated training to a given platform location is derived from a probe trial, in which the platform is removed. Continued searching in the former platform location provides measures of spatial retention. This type of learning has been considered hippocampus-dependent because of its sensitivity to hippocampal lesions (29). The visible platform version of the Morris water maze task can be solved by mice with hippocampal damage, and therefore is used to assess nonhippocampal performance characteristics and elemental learning (29).

The albino NIH Swiss strain was established in 1936 at the National Institutes of Health (NIH; Bethesda, Md.) from outbred Swiss stock obtained from The Rockefeller Institute in 1935 (39).

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Mount Sinai Hospital Research Institute, 600 University Avenue, Toronto, Ontario, M5G 1X5, Canada[1]; Innis College, University of Toronto, Toronto, Ontario, Canada[2]; Department of Molecular and Medical Genetics, University of Toronto, Toronto, Ontario, Canada[3].
[†]Present address: Clinical Psychology Program, Department of Psychology, University of Western Ontario, London, Ontario, Canada.
[*]Corresponding author.

Its pigmented descendent, the Black Swiss strain, was developed by Dr. Carl Hansen at the NIH by a cross of NIH Swiss and C57BL/6N mice, followed by nine generations of backcrossing to NIH Swiss, with selection for the black (*Tyrp1*^{B}) and non-agouti (*a*) alleles. Here we report that both outbred Swiss strains exhibited deficient ability in the Morris water maze task that was severe enough to suggest visual impairment. In addition, NIH Swiss and Black Swiss mice were unable to demonstrate contextual learning. A subsequent polymerase chain reaction (PCR) assay revealed that all of the NIH Swiss and Black Swiss mice included in the experiment were homozygous for the recessive retinal degeneration 1 (*rd1*) mutation of the *Pde6b* gene. Histological examination of NIH Swiss and Black Swiss mouse eyes confirmed the presence of retinal degeneration, which causes visual image blindness by 9 weeks of age (5, 6).

Materials and Methods

Mice. Black Swiss (10 male and 10 female) mice were obtained from Taconic Farms (Germantown, N.Y.), and NIH Swiss (10 male and 10 female) mice were purchased from Harlan Sprague Dawley, Inc. (Madison, Wis.). In addition, mice of the inbred strains C57BL/6J (seven male and six female) and C57BL/6J-*Tyr*$^{c\text{-}2J}$ (B6-*Tyr*$^{c\text{-}2J}$ [19], 12 male and 11 female) were obtained from JAX Research Systems (Bar Harbor, Maine) to act as reference strains. The C57BL/6J strain was selected on the basis that it is the most commonly used host strain for the maintenance and phenotypic testing of mutations and has been shown to perform well in the behavioral paradigms used in the present study. Ocular pigmentation has been shown to correlate strongly with performance in the water maze paradigm (20), so the albino B6-*Tyr*$^{c\text{-}2J}$ strain was chosen to serve as a comparator with regard to potential visual problems associated with the albinism of the NIH Swiss strain.

Husbandry. Mice arrived at our animal facility at 8 weeks of age and immediately were earmarked for identification and housed on corn cob bedding in same-sex, same-strain groups of three to five animals inside individually ventilated, tinted polycarbonate cages, which were changed weekly. The cages were kept inside a single temperature (20 ± 1°C)- and humidity (50 to 60%)-controlled vivarium under a 12:12-h light:dark cycle (lights on, 0700 to 1900), with dry food pellets and sterilized water available ad libitum. After being left undisturbed in their home cage for 9 days of acclimation, subjects were removed from their cages and handled for 2 min on each of five consecutive days. At this time, the dorsal fur of NIH Swiss and B6-*Tyr*$^{c\text{-}2J}$ mice was colored black by the application of a gel-based hair dye (Just For Men, Combe Incorporated, White Plains, N.Y.), the purpose being to make these albino strains clearly visible against the white background of the water maze. Mice were 10 to 12 weeks old at the time of testing, which occurred between 1300 and 1700 h, during the light phase of their cycle.

Based on histological, parasitological, microbiological, and serological screening of sentinel animals from each room, vendor and in-house health surveillance reports indicated that the mice used in the present study were free of adventitious viruses, *Mycoplasma* spp., respiratory and enteric bacteria, ectoparasites, and endoparasites. All animal procedures were approved by the Animal Management Committee of Mount Sinai Hospital and were conducted in accordance with the requirements of the Province of Ontario Animals for Research Act 1971 and the Canadian Council on Animal Care (7, 8).

Morris water maze apparatus. The Morris water maze consisted of a cylindrical tub (diameter, 117 cm; depth, 30 cm) of ivory-colored Perspex (polymethyl methacrylate) that was filled with water (temperature, 26 ± 1°C) to 11 cm below the rim. The water was rendered opaque by the addition of white, nontoxic paint. The pool was divided into four quadrants of equal area, arbitrarily called northeast, southeast, southwest, and northwest. A circular platform (diameter, 10 cm) made of transparent Perspex was submerged 1 cm below the water surface, its center being 30 cm from the perimeter, in the middle of one quadrant (the target quadrant). The platform was covered with white gauze for the purpose of providing a firm grip. The tub was elevated 37 cm above the floor and was surrounded by a wall (~52 cm away) to the north and east, a curtain (~56 cm away) to the west, and a surgical screen (~70 cm away) to the south, behind which the experimenter and home cages were located during trials. Two- and three-dimensional visual cues were positioned around the tub, 64 to 92 cm from its rim. Four 250-W quartz halogen lamps (GE Lighting Canada, Oakville, Ontario, Canada) were positioned on the floor in the four corners of the arena and were aimed at the ceiling to indirectly illuminate the water surface. A closed-circuit television camera was mounted onto the ceiling directly above the center of the pool to convey subject swimming trajectories and parameters to an electronic image analyzer (HVS Image Ltd, Twickenham, Middlesex, UK), which extracted and stored the X-Y coordinates of the subject's position at sample points every 0.01 sec.

Morris water maze procedure. On each day, subjects were moved to the procedure room 30 min prior to testing. Each subject was placed by the tail into the water, immediately facing the perimeter, at one of the cardinal compass points (north, south, east, or west) and then was allowed a maximal time of 90 sec to locate the platform. Finding the platform was defined as staying on it for at least 2 sec; subjects that crossed the platform without stopping (jumping immediately into the water) were left to swim. After staying on the platform for 10 sec, the subject gently was picked up with a steel spatula, returned to its home cage, and allowed to warm up and dry off under a 125-W heat lamp (GE Lighting Canada). If the subject failed to find the platform in the allotted time, it was placed onto the platform for 10 sec and assigned a latency of 90 sec. After each subject in the testing group had completed one trial, the next trial was begun. The first four release points were predetermined to be quasi-random and nonsequential; the last two release points were a repetition of the first two in reverse order. At the end of the experiment, each subject had been released an equal number of times from each point. The entire procedure took four consecutive days, each subject having six training trials per day, with a 20-min intertrial interval.

On the first day (six trials, visible phase), the platform was placed in the southwest quadrant and had a 13-cm high visible cue (1-cm-diameter rod with a ping-pong ball affixed to the top) fitted into a hole at the center, marking its location. For the remaining three days (18 trials, hidden phase), the platform was relocated to the northeast quadrant, where it was hidden from view. A probe trial was administered 20 min after the last trial on the fourth day, when each subject was placed into the water diagonally opposite the target quadrant and allowed 60 sec to search the water, from which the platform had been removed.

Behavioral variables were quantified with the aid of HVS Water 2020 (HVS Image Ltd.), and mean values on each training day were calculated for each subject. Escape performance during training was measured by latency to find the platform (sec). Spatial specificity was measured by duration in the target quadrant (% of total time; chance level, 25%) and duration within 14.5 cm of the perimeter ('thigmotaxis'; % of total time; chance level, 43.5%). Locomotion was measured by swimming speed (cm/s) and duration of floating (% of total time; movement threshold, 5 cm/s). Spatial retention in probe trials was measured by the percentage of total time spent in each quadrant and the number of crossings over the platform location and over equivalent locations in the other quadrants. After the 4-day Morris water maze procedure, mice were given a rest period of 6 days before being tested in contextual fear conditioning.

Contextual fear conditioning apparatus. The fear conditioning apparatus (MED Associates Inc., Georgia, Vt.) consisted of a test chamber (25 cm high by 30 cm wide by 25 cm deep), with a transparent ceiling, front, and back and a removable grid floor of 36 stainless-steel rods (diameter, 3.2 mm; spacing, 4.7 mm) connected to a constant current shock generator. A 12-in. (ca. 30.5 cm), 8-W fluorescent tube (GE Lighting Canada) illuminated the chamber interior through the transparent ceiling, and a white cloth covered the front exterior of the chamber during testing. A personal computer running automated fear conditioning software (FreezeFrame, Actimetrics Software, Evanston, Ill.) administered foot shocks, recorded video images of the chamber, and monitored the activity of subjects throughout the procedure.

Contextual fear conditioning procedure. Immediately prior to context training, the chamber was cleaned with 70% ethanol, which left an odor during training. Each subject was removed from its home cage, placed at the center of the grid floor, and left to explore the test chamber for 2 min prior to conditioning. Activity during this time was recorded as baseline (test-naïve) activity. A single continuous foot shock (1 mA scrambled, 2 sec duration) was administered 2 min 28 sec after the training session started. The subject was removed from the chamber 30 sec later and returned to its home cage.

Approximately 24 h later, each subject was returned to the chamber and monitored for 5 min without the delivery of any foot shock. The activity of each subject was recorded at 0.25-sec intervals by using FreezeFrame automated fear conditioning software (Actimetrics Software), which can detect the minute movements of grooming, sniffing, turning, and rearing. The mean activity during the context exposure was calculated, divided by the mean baseline activity, and used as a measure of contextual learning.

Statistical methods. Subject means for the three days of hidden platform training in the Morris water maze were subjected to a four (strain) by three (day) two-way analysis of variance (ANOVA), with strain as a between-subjects factor and day as a repeated-measures factor. Data from Morris water maze visible platform and probe trials and from contextual fear conditioning tests were subjected to one-way ANOVA between strains. When the ANOVA detected significant strain or day effects, pairwise differences between means for a given variable were evaluated using Tukey's post hoc multiple comparison test, with significance set at $P < 0.05$. ANOVA did not detect sex effects in any of the behavioral tests, so data from male and female mice were pooled and analyzed together. Paired *t* tests were used to compare values measured in different quadrants of the Morris water maze during probe trials and to compare baseline activity versus 24 h context activity in contextual fear conditioning. All statistics were calculated using MINITAB for Windows 13.32 (Minitab Inc., State College, Pa.) and cross-checked against the results obtained independently by using STATISTICA for Windows 5.5 (StatSoft, Tulsa, Okla.). All values reported in the text and figures are expressed as mean ± standard error of the mean (SEM).

Retinal degeneration genotyping. Tail biopsies (1 cm long) were taken from each NIH Swiss (n = 20) and Black Swiss (n = 20) mouse that had been included in the behavioral tests and from single C57BL/6J and B6-*Tyr*$^{c\text{-}2J}$ mice. The tail of a single CBA/J mouse was obtained from Harlan Sprague Dawley, Inc.; this strain was included as a positive control because it is known to be homozygous for *rd1* (24, 45) and has shown impaired spatial learning in the Morris water maze (32). DNA samples isolated from the tail biopsies were subjected to a PCR assay for distinguishing between the mutant (*rd1*) and wild-type (*Pde6b*+) alleles of *Pbe6b* (18), thus identifying three distinct genotypes after agarose gel electrophoresis: *Pde6b*$^+$/*Pde6b*$^+$ (wild type) having a band of 400 bp; *rd1*/*rd1* (homozygous mutant) having a band of 550 bp; and *Pde6b*$^+$/*rd1* (heterozygote) having bands of 400 and 550 bp. To confirm initial findings, the PCR assay was performed on ten additional Black Swiss mouse tails obtained from the breeder (Taconic Farms).

Histological examination of eyes. To verify the findings of the *rd1* PCR assay, 11-week-old NIH Swiss (n = 4) and Black Swiss (n = 4) mice were euthanized for histological examination of their eyes. After CO_2 asphyxiation of the animal, the head was removed and fixed in neutral buffered formalin. The eyes were dissected from the skull and then were processed routinely, sectioned onto slides, and stained with hematoxylin and eosin (H&E). Slides were examined in comparison with sections taken from aged-matched C57BL/6J (*Pde6b*$^+$/*Pde6b*$^+$) and CBA/J (*rd1*/*rd1*) control mice.

Results

Morris water maze. Mean values for each strain in the visible phase (block V) and the hidden phase (blocks 1 through 3) of training are shown in Fig. 1. During the visible phase, there was a significant main effect of strain on latency to find the visible-cued platform (strain: $F_{3,72} = 16.35$, $P < 0.0001$; Fig. 1a). Post hoc comparisons showed that the NIH Swiss and Black Swiss mice had longer escape latencies than did C57BL/6J animals, but only NIH Swiss mice had a longer escape latency than did B6-*Tyr*$^{c\text{-}2J}$ animals. During the hidden phase, the NIH Swiss and Black Swiss mice had longer latencies to find the hidden platform than did C57BL/6J and B6-*Tyr*$^{c\text{-}2J}$ animals (strain: $F_{3,216} = 53.52$, $P < 0.0001$; day: $F_{2,216} = 5.82$, $P < 0.005$; strain * day interaction: $F_{6,216} = 1.05$, $P > 0.05$; Fig. 1a). The outbred Swiss strains had escape latencies that remained unchanged throughout training, despite swimming faster than C57BL/6J and B6-*Tyr*$^{c\text{-}2J}$ mice (strain: $F_{3,216} = 57.69$, $P < 0.0001$; day: $F_{2,216} = 0.11$, $P > 0.05$; strain * day interaction: $F_{6,216} = 0.42$, $P > 0.05$; data not shown). A high level of thigmotaxis was maintained by the NIH Swiss and Black Swiss animals throughout training. In contrast, the B6-*Tyr*$^{c\text{-}2J}$ and C57BL/6J mice had intermediate and low levels of thigmotaxis, respectively, which decreased with training (strain: $F_{3,216} = 127.07$, $P < 0.0001$; day: $F_{2,216} = 2.71$, $P > 0.05$; strain * day

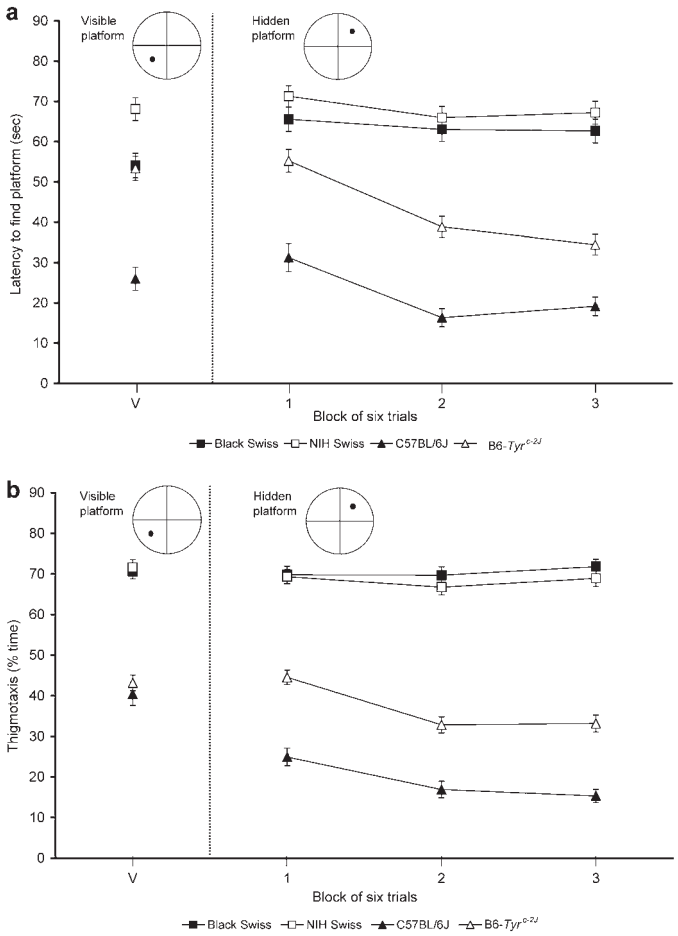

Figure 1. Morris water maze training. Mean value (± SEM) of Black Swiss (closed squares n = 20), NIH Swiss (open squares, n = 20), C57BL/6J (closed triangles, n = 13), and B6-*Tyr*$^{c\text{-}2J}$ (open triangles, n = 23) mice for (A) latency (sec) to find the platform and (B) thigmotaxis (% of time within 14.5 cm of the perimeter) at each block of six training trials in the Morris water maze with a visible platform in the southwest quadrant (block V) and a hidden platform in the northeast quadrant (blocks 1 through 3).

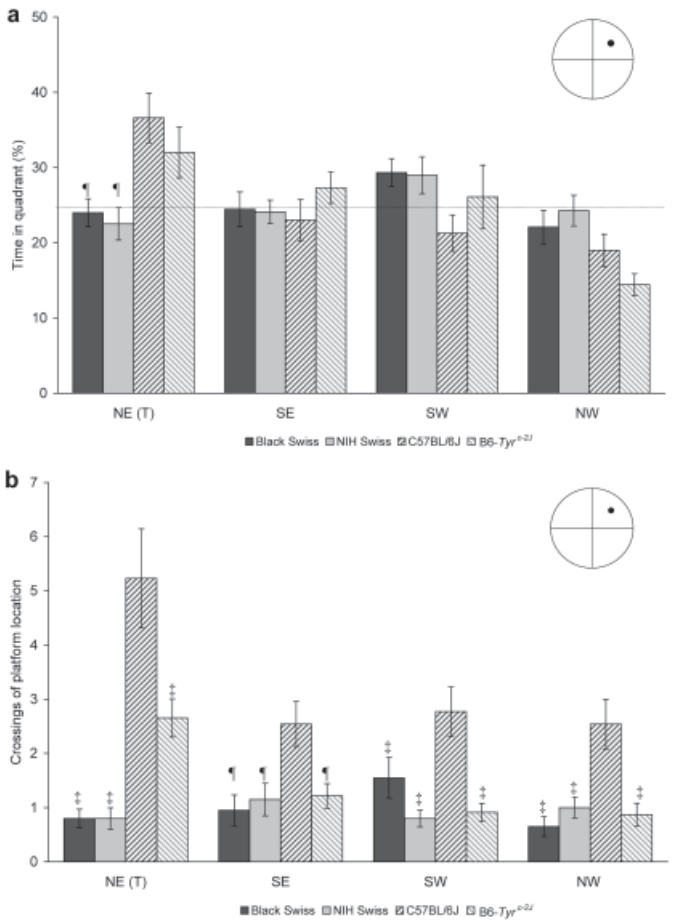

Figure 2. Morris water maze probe trial. Mean measurements (± SEM) of spatial retention for the Black Swiss (dark shaded columns, n = 20), NIH Swiss (light shaded columns, n = 20), C57BL/6J (dark hatched columns, n = 13), and B6-*Tyr*$^{c\text{-}2J}$ (light hatched columns, n = 23) mice in the memory probe trial in the Morris water maze. (A) Percentage of total time spent in the southeast (SE), southwest (SW) and northwest (NW) quadrants and in the trained (T) northeast (NE) quadrant. (B) Number of crossings over the platform location in the trained (T) northeast (NE) quadrant and over equivalent locations in the other quadrants. Symbols indicate a significant difference ($^{¶}P < 0.01$; $^{‡}P < 0.0001$) from the C57BL/6J reference strain.

interaction: $F_{6,216} = 1.01$, $P > 0.05$; Fig. 1b). There was no difference between the Black Swiss, NIH Swiss, and C57BL/6J mice in percentage of time spent floating, but B6-*Tyr*$^{c\text{-}2J}$ mice floated for a longer time throughout training (strain: $F_{3,216} = 12.72$, $P < 0.0001$; day: $F_{2,216} = 0.08$, $P > 0.05$; strain * day interaction: $F_{6,216} = 0.46$, $P > 0.05$; data not shown).

In the probe trial, there was a significant main effect of strain on time spent in the target (T) quadrant (strain: $F_{3,72} = 5.11$, $P < 0.005$; Fig. 2a) and on the number of crossings of the platform location (strain: $F_{3,72} = 22.04$, $P < 0.0001$; Fig. 2b). Post hoc comparisons revealed that B6-*Tyr*$^{c\text{-}2J}$ and C57BL/6J animals crossed the platform location more frequently than did mice of the outbred Swiss strains, with C57BL/6J animals also spending more time in the target quadrant. Paired *t* tests comparing the target quadrant with the mean of the alternative quadrants showed that mice of the C57BL/6J and B6-*Tyr*$^{c\text{-}2J}$ strains had significant ($P < 0.05$) spatial biases for the target quadrant, whereas the NIH Swiss and Black Swiss mice spent approximately 25% of total time in each quadrant and did not cross the platform location more frequently than equivalent platform locations in the other quadrants.

Contextual fear conditioning. Prior to the delivery of the foot shock, baseline (test-naïve) activity was not affected by strain, and each strain had activity levels > 99% (strain: $F_{3,72} = 1.76$, $P > 0.05$; data not shown). There was, however, a significant main effect of strain on contextual learning (strain: $F_{3,72} = 30.09$, $P < 0.0001$; Fig. 3a). Paired *t* tests showed that the C57BL/6J and B6-*Tyr*$^{c\text{-}2J}$ animals suppressed their activity in the 24-h context test relative to baseline activity ($P < 0.005$), whereas the NIH Swiss and Black Swiss mice exhibited no difference in activity.

Retinal degeneration genotyping. After the behavioral tests, all of the outbred Swiss mice and the single C57BL/6J, B6-*Tyr*$^{c\text{-}2J}$, and CBA/J mice were genotyped at the *Pde6b* locus to test for the presence of the *rd1* allele that confers retinal degeneration. Representative PCR products after agarose gel electrophoresis are shown in Fig. 4. Initial experiments showed that all of the NIH Swiss (n = 20) and Black Swiss (n = 20) mice tested were homozygous for *rd1*. The apparent fixation of the *rd1* allele

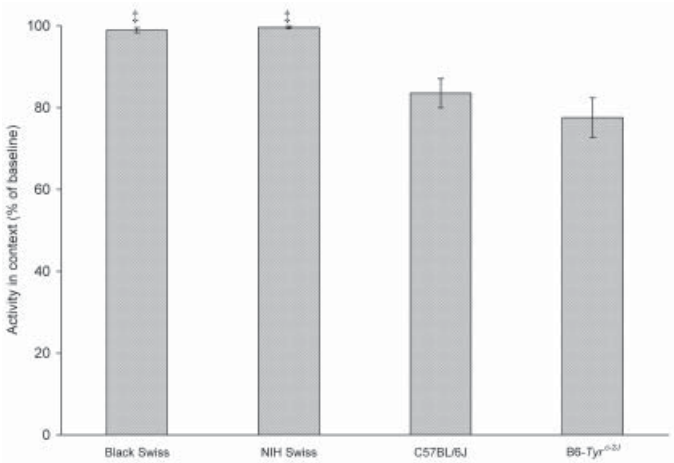

Figure 3. Contextual fear conditioning. Activity of Black Swiss (n = 20), NIH Swiss (n = 20), C57BL/6J (n = 13), and B6-*Tyr*$^{c\text{-}2J}$ (n = 23) mice in the context is shown as the percentage of baseline (test-naïve) activity during training (mean ± S.E.M.), a low value being indicative of contextual learning. The symbol indicates a significant difference ($^{\ddagger}P < 0.0001$) from the C57BL/6J reference strain.

within the Black Swiss colony at Taconic Farms was confirmed when ten additional Black Swiss mice also were found to be homozygous for *rd1*.

Histological findings. The retinas of four NIH Swiss and four Black Swiss mice were examined and displayed a histological appearance typical of mice homozygous for the *rd1* mutation. The NIH Swiss and Black Swiss retinas were markedly thinner than those of C57BL/6J (*Pde6b*$^{+}$/*Pde6b*$^{+}$) control mice, lacked outer plexiform and outer nuclear layers, and had no discernable photoreceptor outer segments (Fig. 5).

Discussion

In the Morris water maze, NIH Swiss and Black Swiss mice had long escape latencies, which did not decrease with further training, whereas the escape latencies of C57BL/6J and B6-*Tyr*$^{c\text{-}2J}$ mice were shorter and decreased with additional trials. In the probe trial, with the platform removed, C57BL/6J and B6-*Tyr*$^{c\text{-}2J}$ mice demonstrated a selective search strategy directed towards the trained NE quadrant. NIH Swiss and Black Swiss mice, in contrast, demonstrated a random search strategy, spending equivalent amounts of time in each quadrant of the pool. The performance characteristics of the NIH Swiss and Black Swiss strains in the Morris water maze thus were reminiscent of those reported for the outbred Swiss-derived ICR strain (1, 16) and the inbred Swiss-derived FVB and SJL strains (33, 34, 40, 44), all of which are known to have retinal degeneration (23, 24, 26, 36, 42, 45).

In the fear conditioning test, because the contextual cues are not only visual, we anticipated that the possible visual impairment of the NIH Swiss and Black Swiss strains, as suggested by their water maze performance, would not be grossly debilitating. However, upon exposure to the context, NIH Swiss and Black Swiss mice showed a level of activity that was not different from preshock baseline, suggesting that they were unable to perceive and remember multiple cues from the context. In contrast, C57BL/6J and B6-*Tyr*$^{c\text{-}2J}$ mice demonstrated contextual learning by suppressing their activity when re-exposed to the context.

The apparent failure of the NIH Swiss and Black Swiss strains

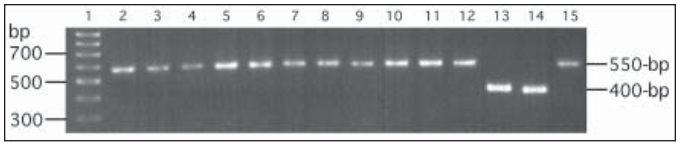

Figure 4. PCR assay for genotyping the *Pde6b*^{rd1} mutation. Lane 1, 0.5 µg 100-bp DNA ladder (300-bp, 24.5 ng; 500-bp, 82.0 ng; 700-bp, 57.5 ng); lanes 2 through 6, NIH Swiss mice; lanes 7 through 12, Black Swiss mice; lane 13, C57BL/6J mouse; lane 14, B6-*Tyr*$^{c\text{-}2J}$ mouse; lane 15, CBA/J mouse (*rd1* homozygous positive control). *Pde6b*$^{+}$ (wild-type) mice have a single band of 400 bp, and *rd1*/*rd1* (homozygous mutant) mice have a single band of 550 bp. The sizes of the DNA ladder fragments and PCR products are indicated on the sides of the figure.

to demonstrate contextual learning, as evidenced by the absence of activity suppression (freezing), is consistent with similar findings reported for the Swiss-derived outbred strains ICR and CD-1 (1, 16). The impairment of the CD-1 strain, which is wild-type at the *Pde6b* locus (42) and has demonstrated good spatial learning in the water maze (1, 14, 16, 17), suggests that outbred Swiss mice have a contextual fear conditioning performance deficit that is unrelated to retinal degeneration. Because there is heritable variation among mice in the strategies they use to cope with environmental demands (2), this impaired freezing may represent a particular motor or motivational characteristic of outbred Swiss-derived mice, which may be noncognitive (1). Whatever the reason, it is clear that outbred Swiss mice, regardless of *Pde6b* genotype, may not be the best choice for fear conditioning experiments.

The poor performance of the NIH Swiss and Black Swiss mice in the Morris water maze led us to suspect that they might have retinal degeneration. This suspicion was strengthened when a subsequent PCR assay for the *rd1* mutation of *Pde6b* showed that all of the NIH Swiss and Black Swiss mice subjected to behavioral tests, plus ten additional Black Swiss mice, were homozygous for *rd1*. These findings are consistent with the assumption that the *rd1* mutation is fixed within the Black Swiss colony at Taconic Farms and the NIH Swiss colony at Harlan Sprague Dawley.

Histological examinations of NIH Swiss and Black Swiss retinas provided conclusive evidence of retinal degeneration in these strains. Given the known high incidences of retinal degeneration in several other Swiss-derived strains, namely the outbred ICR (23, 26, 36, 42), Crl:CFW(SW)BR, and Tac:(SW) (42) strains and the inbred FVB, NIH, SJL, and SWR strains (12, 24, 43, 45), it is unsurprising that NIH Swiss and Black Swiss mice also are affected. Furthermore, a previous study reported a lower-than-expected frequency of *rd1* heterozygotes in other Swiss-derived outbred strains (42). Although these strains are bred by their respective suppliers to maintain maximal heterozygosity, there is very strong selection (albeit inadvertent) for *rd1* homozygosity in any mixed colony in which the *rd1* allele was present originally (35, 42). Visually impaired mice are more likely to be taken out of the cage first and used for breeding because their normally sighted cagemates are more adept at perceiving danger and thus evade capture.

Retinal degeneration can be a major confounder of experimental data in ophthalmic toxicity tests (42) and in behavioral tests that require visual acuity, as has been shown for three Alzheimer's transgenic mouse lines (15). As such, reports of behavioral abnormalities in pharmacologically treated NIH Swiss mice and

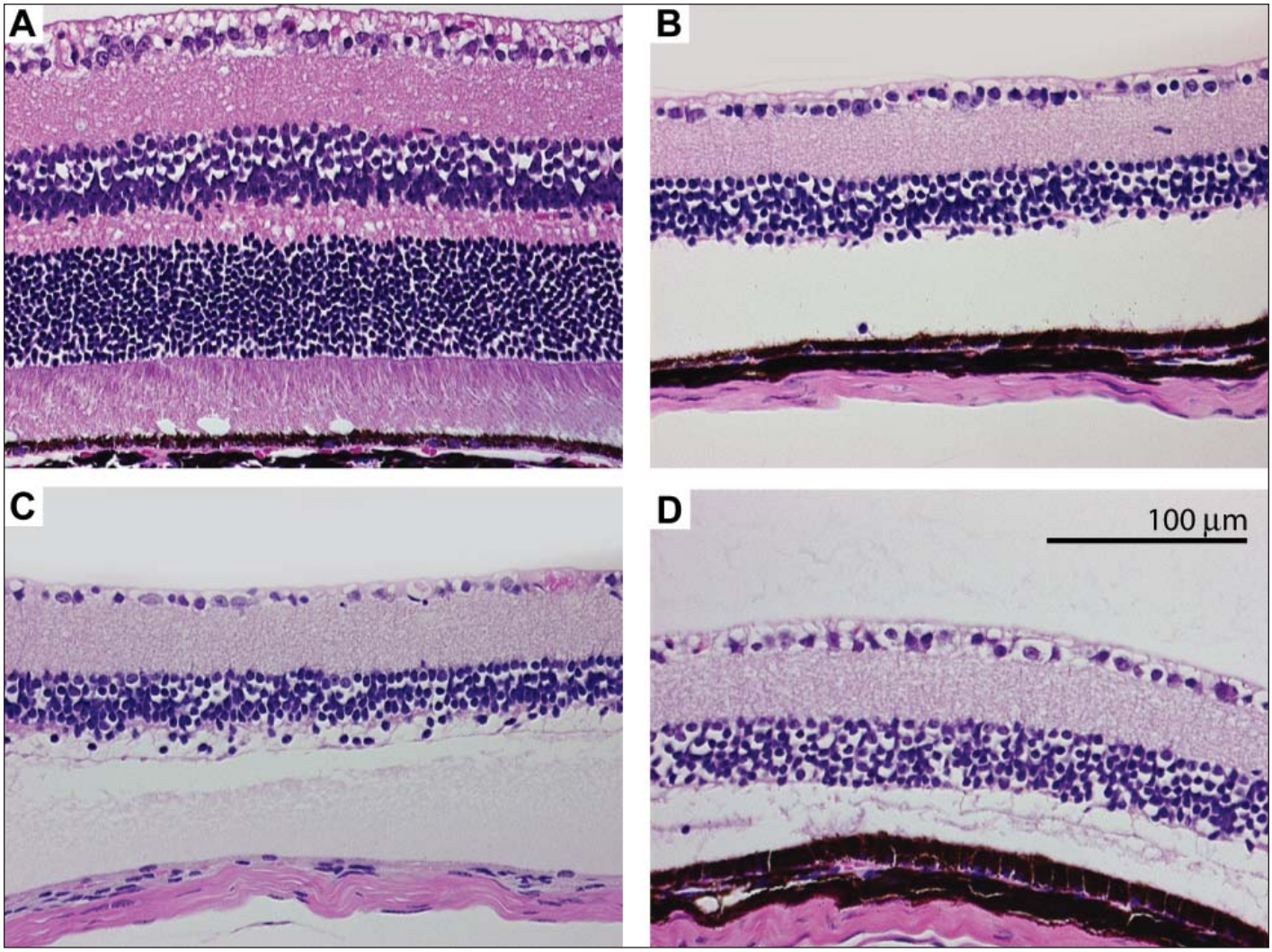

Figure 5. Photomicrographs of representative sections of retinas from 11-week-old mice. (A) Normal control C57BL/6J (*Pde6b*$^{+}$/*Pde6b*$^{+}$) retina. (B) Affected control CBA/J (*rd1*/*rd1*) retina. (C) NIH Swiss retina. (D) Black Swiss retina. Retinas of affected mice (B through D) are markedly thinner than those of normal mice (A) and lack outer plexiform, outer nuclear, and photoreceptor outer segments. H&E stain.

in null mutants with a Black Swiss genetic background should perhaps be re-evaluated in light of the finding that these strains have retinal degeneration. For example, mice carrying targeted mutations of the *Adcy8* and *Pafah1b1* genes are reported to have reduced anxiety in the elevated plus maze (41) and impaired spatial learning in the water maze (37), respectively. However, these mutations were maintained on 129/Sv x Black Swiss segregating backgrounds, with which one would expect 25% of mice to be homozygous for *rd1* (28). In such cases, when one or more of the progenitor strains is known to harbor the *rd1* allele, it is important to genotype mice for both the presence of the targeted mutation and the absence of *rd1* in the homozygous state to avoid confounds in behavioral data analyses.

We conclude that NIH Swiss and Black Swiss mice have retinal degeneration caused by homozygous inheritance of the *rd1* mutation, which leads to severe impairment in behavioral tests that are dependent on visual acuity. This finding should help investigators make an informed choice when deciding which mouse strain will be most suitable for the hypothesis they are trying to test.

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