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Behavioral validation of the Ts65Dn mouse model for Down syndrome of a genetic background free of the retinal degeneration mutation *Pde6b^{rd1}*

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Abstract

The Ts65Dn mouse is the most studied and complete aneuploid model of Down syndrome (DS) widely available. As a model for human trisomy 21, these mice display many attractive features, including performance deficits in different behavioral tasks, alterations in synaptic plasticity and adult neurogenesis, motor dysfunction, and age-dependent cholinergic neurodegeneration. Currently, Ts65Dn mice are maintained on a genetic background that leads to blindness in about 25% of their offspring, because it segregates for the retinal degeneration 1 (*Pde6b^{rd1}*) mutation of C3H/HeSnJ. This means that 25% of the mice have to be discarded in most experiments involving these animals, which is particularly problematic because the Ts65Dn stock has low reproductive performance. To circumvent this problem, we have bred the Ts65Dn extra chromosome many generations into a closely related genetic background that does not carry the *Pde6b^{rd1}* mutation. Although the new genetic background is expected to be nearly identical to the original, differences in genetic background have the potential to alter mouse performance in certain behavioral tests. Therefore, we designed the present study primarily as a behavioral validation of Ts65Dn mice of the new background. We compared side-by-side their performance with that of Ts65Dn mice of the original background on the following set of assessments: 1) body length and weight; 2) 24-hour locomotor activity; 3) the Morris water maze; 4) fear conditioning; and 5) grip strength. Except for very subtle differences on water maze performance, we found no significant differences between Ts65Dn mice on the two backgrounds in the measures assessed.

Keywords

Down syndrome; Ts65Dn mice; aneuploidy; trisomy 21; trisomy 16; mouse models; Morris water maze; fear conditioning

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Introduction

The Ts(17¹⁶)65Dn segmental trisomy mouse model (designated Ts65Dn) was generated two decades ago at The Jackson Laboratory (JAX) as the first postnatal-viable aneuploid mouse model for Down syndrome (DS) [1]. Since that time, several other aneuploid mouse models for DS have been produced [2,3,4,5]. At present, however, the Ts65Dn mouse is still the most complete mouse model for DS that is widely available to the scientific community. In addition, the more than 150 PubMed-indexed publications (as well as more than a dozen book chapters and other non-indexed publications) that have been published about research on this mouse, makes it, by far, the most extensively studied animal model for DS. Although Ts65Dn mice do not present all the features associated with DS (for example, the characteristic age-related pathology indistinguishable from Alzheimer's disease), these animals display a remarkably diverse array of DS phenotype-analogs. These include significant learning deficits in specific behavioral tasks, craniofacial dysmorphogenesis, motor dysfunction, and age-dependent loss of cholinergic markers in basal forebrain cholinergic neurons [6,7,8,9]. Recently, even congenital vascular and intracardiac defects similar to those found in 40–50% of all individuals with DS have been reported in 17% of Ts65Dn neonates [10].

Findings from studies on Ts65Dn mice also have had a significant predictive value for DS. It was the finding of deficits in putatively hippocampus-dependent tasks in these mice that led to the inclusion of hippocampus-dependent measures on a comprehensive neuropsychological battery applied to individuals with DS [11]. This pivotal work revealed a disproportionately large performance deficit in hippocampus-dependent measures by persons with DS compared to typically developing individuals. In addition, reduction in granule cell density in Ts65Dn mice has predicted correctly an analogous pathology in human beings [12].

Soon after the Ts65Dn mouse was generated, it became clear that backcrossing the Ts65Dn chromosome onto any of the commonly available inbred genetic backgrounds was not possible because of failure to recover trisomic progeny (reviewed in [6]). Therefore, these animals have been maintained by repeated backcrossing of Ts65Dn females to C57BL/6JEi×C3H/HeSnJ (B6EiC3Sn) F1 hybrid males to minimize genetic segregation and genetic drift. Still, it is true that each locus at which B6Ei and C3Sn differ can be either heterozygous or homozygous for the allele from either parental strain. This means that homozygosity for the C3H-derived retinal degeneration mutation *Pde6b^{rdl}* (which leads to blindness within a few weeks after birth) occurs in an average of 25% of Ts65Dn offspring. Therefore, for most experiments, all Ts65Dn mice must be tested for vision or genotyped at the *Pde6b^{rdl}* gene. Such procedures increase significantly colony maintenance costs and the resulting culling of blind mice decreases significantly the yield of useful animals from Ts65Dn breeding colonies.

In the present study, we describe a new stock of Ts65Dn mice that does not develop retinal degeneration. Given the vast literature on these animals and given the genetic background-dependence of certain behavioral phenotypes (see for example [13]), we have been especially careful in minimizing changes in genetic background in developing this new Ts65Dn stock. To this end, we have produced Ts65Dn mice by repeated backcrossing of Ts65Dn females to B6EiC3H F1 hybrid males derived from a new congenic strain of C3H mice (C3Sn.BLiA-*Pde6b⁺*/Dn; JAX Stock No. 3648), which lacks the blindness-causing recessive mutant allele. We produced this C3H congenic by backcrossing the wild type allele of *Pde6b* from C3A.BLiA-*Pde6b⁺*/J for 10 generations to C3H/HeSnJ, the C3H substrain used to make the F1 hybrid in the original Ts65Dn stock. The use of this new Ts65Dn stock, named B6EiC3Sn.BLiA *a/a* -Ts65Dn (abbreviated to B6C3.B-Ts65Dn here for simplicity), has the potential to increase the production of usable Ts65Dn mice up to 25%. Here, we report the results of comparing side-by-side Ts65Dn mice of the new stock with mice of the standard Ts65Dn stock (B6EiC3Sn *a/a*-Ts65Dn; Stock No. 1924) using several assessments (mostly

behavioral) that were aimed at providing initial validation to the use of this new Ts65Dn stock. These assessments consisted of: 1) measurements of body length and weight; 2) quantification of 24-hour locomotor activity; 3) Morris water maze performance; 4) fear conditioning performance; and 3) grip force measurements.

Materials and Methods

Mouse Breeding and Care Procedures

All mice were produced at The Jackson Laboratory (JAX) and shipped to the University of Colorado Denver School of Medicine at age 2–3 months. The Ts65Dn stock homozygous for the wild type allele for retinal degeneration 1 (*Pde6b*⁺) was made by backcrossing the C57BL/6-derived *Pde6b*⁺ allele from JAX strain C3A.BLiA-*Pde6b*⁺/J (Stock Number: 001912) 10 generations (N10) onto C3H/HeSnJ (Stock Number: 000661), genotyping at each generation by PCR for the *Pde6b*⁺ allele. The JAX 001912 strain was developed in the laboratory of Willem J. de Grip at Erasmus Universiteit, Rotterdam, the Netherlands. The *Pde6b*⁺ allele from C57BL/LiA was backcrossed 9 generations onto C3Hf/HeA followed by intercrossing heterozygous progeny of the N9 generation to obtain homozygous wild type (*Pde6b*⁺/*Pde6b*⁺) mice. This congenic strain had been maintained by more than 30 generations of sibling mating when it was imported into The Jackson Laboratory in 1991 ([14] and JAXStrain, <http://jaxmice.jax.org/strain/001912.html>). Heterozygous N10 mice from our backcrosses to C3H/HeSnJ were intercrossed to produce the *Pde6b*⁺/*Pde6b*⁺ homozygous congenic strain C3Sn.BLiA-*Pde6b*⁺/Dn (Stock Number: 003648). Mice of this congenic strain are mated to C57BL/6JeiJ (Stock Number: 000924) to produce an F1 hybrid (B6EiC3Sn.BLi, Stock Number 003647) that is expected to be genetically identical to the F1 hybrid used for the current standard Ts65Dn stock (B6EiC3Sn *a/A*-Ts65Dn, Stock Number 001924; hereafter B6C3-Ts65Dn for simplicity), except that none of the mice carry the *Pde6b*^{rd1} mutation. Before comparison of this new stock with the existing Ts65Dn stock, we crossed Ts65Dn females for 5 generations to males of the new F1 hybrid. The new Ts65Dn stock is named B6EiC3Sn.BLi *a/A*-Ts65Dn (Stock Number: 005252; abbreviated here as B6C3.B-Ts65Dn).

While at JAX, mice were housed in a conventional mouse room in the Research Animal Facility with a controlled light cycle of 14 light:10 dark, NIH31 6% fat diet (Purina) and acidified water *ad libitum*, pine shavings bedding and clean cages and water weekly. All mice were genotyped for the Ts65Dn chromosome using the quantitative polymerase chain reaction (qPCR) method developed by [15]. In addition, mice from the current standard Ts65Dn stock were also PCR-screened for retinal degeneration due to *Pde6b*^{rd1} homozygosity [16], and only animals free of retinal degeneration were used in this study. All breeding and genotyping procedures were approved by JAX's Animal Care and Use Committee (IACUC). JAX adheres to the "Principles of laboratory animal care" (NIH publication No. 86-23, revised 1985).

For the behavioral assessments described in this study, standard B6C3-Ts65Dn and the new B6C3.B-Ts65Dn mice, and their respective euploid littermate controls were shipped to and maintained at the Center for Laboratory Animal Care at the University of Colorado Denver School of Medicine on a 12:12 h light/dark cycle with *ad libitum* access to food (also NIH31 6% fat diet) and water. Animals were housed with their littermates until a week before experimental tests and then singly housed after that. Only male animals were used in experiments because the vast majority of experiments published to date have been performed on male mice. The total number of mice per genotype used in the experiments described here were 46 standard Ts65Dn, 46 standard euploid control mice, 46 B6C3.B-Ts65Dn, and 44 euploid control mice of the new genetic background. The age of the animals varied between 4 and 5 months. To avoid the artifacts due to effects of training history in behavioral test batteries, we divided the animals into three groups, according to the experimental procedures in which they were tested. These groups consisted of: 1) mice tested for fear conditioning performance,

from which we also measured body length and weight; 2) mice used in the quantification of 24-h locomotor activity, which were subsequently tested in the Morris water maze; and 3) mice used in grip force measurements. The exact numbers of animals used in each experiment can be found in figure legends. All behavioral assessments were performed in accordance with the "Principles of laboratory animal care" (NIH publication No. 86-23, revised 1985) and under the approval of the University of Colorado Denver's IACUC.

Experimental Procedures

Measurements of body length and weight—Mouse body weight was assessed with a digital balance with dynamic weighting feature, which was set to calculate the mean value of the weighing results over a 3-s time interval to minimize body movement artifacts (Mettler Toledo PB1501-S). Distances from the nose to tail base and from nose to the end of the tail were measured with a standard metric caliper. Also, to avoid measurement artifacts due to body movement during manual restraint, we performed these linear distance measurements in mice anesthetized under deep halothane anesthesia at the end of the behavioral tests.

Quantification of 24-hour locomotor activity—In the present study, we performed 24-h automatic assessments of spontaneous locomotion of mice from the two genotypes and two genetic backgrounds. Activity data were obtained from photocell arrays especially adapted to the exterior walls of the mouse home-cages (Opto-Max Activity Meter, Columbus Instruments, Columbus, OH). These photocell arrays were mounted in a one-dimensional-with-rearing configuration, which consisted of two horizontal rows of 16 photocells (beam spacing = 2.54 cm) vertically offset from each other by approximately 5 cm. The following five experimental measures (outputted by the Opto-Max v2.28-B software) were analyzed in the present study: (1) total activity, (2) ambulatory activity, (3) rearings, and (4) horizontal and (5) vertical stereotypic activities. Total activity refers to the sum of broken beam counts within the two axes of monitoring (horizontal and vertical). Ambulatory activity is the count of different beams broken in the horizontal axis, which ignores repeated beam breaks (stereotypic movement) associated with scratching or grooming. Rearing activity is the number of valid beam breaks in the rearing plane demarcated by the second horizontal rows of photocells. In order to be considered a valid rearing count, prior to the beam break in the rearing plane, all of the beams in the rearing plane must have been detected as unbroken for a minimal amount of time equal to the "plane break delay" (defined as 1-s in the present study). Horizontal and vertical stereotypic activities refer to the number of bursts of stereotypic activity that the animal engages in the horizontal and vertical planes during the 24-h monitoring. A stereotypic burst is recorded upon the first detection of repeated interruption of the same beam. As long as stereotypic counts are detected within the "burst terminator time" (also defined as 1-s in the present study), it is counted as a single stereotypic burst. If a count is detected after the burst terminator time expires, then the next stereotypic event will start and another stereotypic burst will be recorded.

Water Maze task—The experimental protocol was based on Stasko and Costa [17]. We used a temperature-regulated circular swim tank (95-cm in diameter and 17-cm deep). A small platform (6.5-cm diameter circle, 1-cm below the surface of the water) was placed into the tank in the geometric center of one of four quadrants, and a computer-assisted video-tracking device system was used to monitor and quantify the animals' performance (Videomex-V, Columbus Instruments, Columbus OH). The water temperature was maintained at 22 ± 0.5 °C, which preliminary tests suggested was "high enough" to reduce stress and potential hypothermia, but was "low enough" to maintain the animals' motivation to escape the pool and reduce floating behavior, an issue with Ts65Dn mice.

The following test sequence was used: "free swimming" (1 trial) → hidden platform (9 blocks of 4 trials) → 1 probe trial → visible platform (4 blocks of 4 trials) → 1 probe trial → reverse

platform (6 blocks of 4 trials) → 1 probe trial. “Free swimming” and probe trial were trials in which no platform was present in the water tank and the swimming trajectories were analyzed for 60 seconds. In the hidden platform portion of the test, the location of the submerged escape platform was not marked by any immediate cues. The visible platform portion of the test is used as a control to assess the animals’ gross ability to see the presence of a conspicuous flag signaling the platform’s position. Finally, in the reverse platform portion of the test, the hidden platform position was moved to the opposite (reverse) side of the tank. Animals were tested in two blocks of trials per day, with a block defined as a set of four swimming trials in which the mice were placed in the tank from one of four randomly assigned positions. Trials were separated from each other by a period of 15–20 min (to avoid hypothermia), during which time the mice were placed in their home cage under a heat lamp. Once the platform was located, mice were allowed to remain on the platform for approximately 5-s. When the mice did not find the platform, they were allowed to search the tank for a maximum of 60-s, after which time they were gently guided to the platform and also allowed to remain on top of it for 5–10-s.

The dependent measures assessed were latency to locate the platform and escape from the water on each trial, percentage of time spent in each quadrant, number of crossings over the trained platform location, and percentage of time in the periphery of the water tank (thigmotactic behavior). Given that the mean speed of Ts65Dn mice is not significantly different from that of euploid control animals, information on distance travelled was considered redundant and not included in the present study. Also, because the probe trial results, different from the latency data, are not the average of four trials, which tends to reduce artifacts due to floating behavior during a single trial, we excluded data from mice that spent more than 90% floating during any given probe trial, which represented less than 10% of the animals in any given experiment. (Floating behavior seems to be an acquired strategy, given that during the initial probe there was virtually no floating behavior.) These measures were obtained from the direct or processed output of the Videomex water maze program (for more details, see [17]). All experiments were videotaped for archival purposes.

Contextual fear conditioning—Here, we tested mice of two genotypes and two genetic backgrounds with a two-pairing fear conditioning protocol adapted from Dineley et al. [18] and Sananbenesi et al. [19]. Four groups comprising the two genotypes and two genetic backgrounds were used, totaling 46 animals. Mice were placed in the fear conditioning chamber (Med Associates, St. Albans, VT, Modular Mouse Test Chamber), which represented the contextual-conditioned stimulus (CCS), for a total of 212 s. Animals were left free to explore the chamber for 180-s. Then, a 30-s acoustic-conditioned stimulus (ACS; white noise, 80 dB) was delivered. At the end of the ACS, a mild, but unpleasant electric shock (the unconditioned stimulus, or US; 2 s, 0.7 mA, constant electric current) was applied a single time to the grid floor. In the first stage of testing, we evaluated contextual fear learning by returning the animals to the training context 24-h post-training, and freezing behavior was scored for 180-s, every 9-s by two trained observers aware of the mouse genotypes. (We have not attempted to blind the experimenters to genotype because, typically, Ts65Dn mice can be identified by a trained observer, with a reasonable degree of accuracy, by simple visual inspection.) Freezing behavior is defined as a species-specific defensive reaction characterized by lack of movement other than respiration and heartbeat, associated with crouching posture [20,21] and was used as an indicator of learning. In the second stage of the test, we evaluated cued fear learning by placing the animals in a different context (novel odor, lighting, cage floor, and visual cues) following contextual testing. Baseline behavior was scored by the observers for 180-s, every 9-s, which was followed by the ACS for a period of 180-s (again, scored every 9-s). The dependent measures assessed in this assay were the mean number of observations indicating freezing behavior, expressed as percentage of freezing in relation to the total number of observations

for each stage of the test. All experiments were videotaped for offline scoring and archival purposes.

Grip force measurements—The production of mouse forelimb grip forces was assessed with the procedure first described by Meyer et al. [22] and according to modifications introduced by Costa et al. [23]. These modifications include two basic changes to the original procedure to improve the reproducibility of the measurements. First, the grasping ring was covered with fine-grain sand paper to increase grasp friction and set vertically to motivate the animals to grasp to the ring more consistently. Second, a gravity-driven system was used to produce a consistent 10 N downward force onto the animal's tail. The system is manually triggered by the experimenter when he/she determines that the mouse has a firm hold onto the grasping ring of a digital push-pull strain gauge (Grip Strength Meter, Columbus Instruments), which causes the mouse to fall on a soft cushion about 25 cm below the grasping ring. Dynamic information on the grip force production was obtained by processing the digital signals originating from the strain gauge and fed into a high impedance input of an instrumentation amplifier (Model 440, Brownlee Precision, Santa Clara, CA). Amplified signals were low-pass filtered (500 Hz) and digitized (at 2 kHz) into a microcomputer with a data acquisition board and software (Digidata 1322A and PCLAMP 9, Molecular Devices Corporation, Union City, CA) and analyzed offline. The primary measures of these experiments were peak grip force (in grams) and relative grip force rise slope (assessed from 10% to 90% of the peak grip force, and expressed in % of the peak grip force / millisecond).

Statistics

Dependent measures of each experiment were expressed as mean $\pm$ SEM for each group of mice of the same genotype/genetic background. Simple comparisons between performance data of mice from the two genotypes and the two genetic backgrounds were performed by two-way analysis of variance (ANOVA), and *post hoc* multiple comparisons were done by Fisher protected least significant difference (PLSD) test (Statistica, StatSoft, Tulsa, OK). Performances across multiple blocks of trials between genotypes and genetic backgrounds were compared with multivariate repeated measures ANOVA, and *post hoc* multiple comparisons were done by Fisher's PLSD test (also with Statistica). In all figures, statistically significant differences between group means is expressed generally as *, **, ***, for $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. In Figure 4, the symbols † and †† are used to denote statistically significant differences in mean latency between the B6C3.B-Ts65Dn group and its euploid control group ($p < 0.05$ and $p < 0.01$, respectively). Finally, a single asterisk is used in the text to denote significance.

Results

The body weight and length of Ts65Dn mice and their euploid controls are not significantly affected by the new genetic background

We have found a significant genotype-dependence ($F_{1,58} = 26.757$, $*P < 0.001$), but no significant background-dependence ($F_{1,58} = 0.000$, $P = 0.995$) or background $\times$ genotype interaction ($F_{1,58} = 0.508$, $P = 0.479$) for the mean body weight of the animals used in this study. Accordingly, *post hoc* analyses revealed that the mean body weight of standard control euploid mice is greater than this measure in Ts65Dn mice ($*P < 0.001$). Also, the mean body weight of control euploid mice in the new genetic background is greater than the mean body weight of B6C3.B-Ts65Dn mice ($*P = 0.002$). These results are depicted in Fig. 1A.

We also detected a significant genotype-dependence for mean body length measured from nose to base ($F_{1,58} = 15.20$, $*P < 0.001$) and from the nose to the tip of the tail ($F_{1,58} = 49.86$, $*P < 0.001$). No significant background-dependence or background $\times$ genotype interaction was

detected for the mean body length measured from nose to base ($F_{1,58} = 0.82$, $P = 0.369$; $F_{1,58} = 2.64$, $P = 0.110$; respectively) and from the nose to the tip of the tail ($F_{1,58} = 0.00$, $P = 0.945$; $F_{1,58} = 1.07$, $P = 0.306$; respectively). *Post hoc* analyses of body length measurements showed that mean nose-to-base distance assessed in standard control euploid mice was significantly longer than in Ts65Dn mice ($*P < 0.001$; Fig. 1B). In our sample, however, the difference in mean nose-to-base length between control and Ts65Dn mice in the B6C3.B background failed to reach significance ($P = 0.107$; Fig. 1B). Mean nose-to-tail measurements are represented in Fig. 1C. In relation to this measure, we found that euploid control mice in both the standard B6C3 and the B6C3.B background were significantly longer than their respective Ts65Dn counterparts ($*P < 0.001$ for both *post hoc* comparisons).

Ts65Dn in the new genetic background display nocturnal hyperactivity comparable to that seen in the original Ts65Dn stock

Reeves et al. [26] have shown that Ts65Dn mice display increased activity levels during the dark phase of a 12/12-h light/dark cycle. To analyze whether the B6C3.B-Ts65Dn mice retain this phenotype we performed 24-h automatic assessments of spontaneous locomotion of mice of the two genotypes and two genetic backgrounds.

Fig. 2 depicts mean total activity in one hour as a function of time. In this figure, time = 0 means the beginning of the “dark cycle” (i.e. lights off at 7:00 PM). Fig. 2.A represents the total activity of Ts65Dn mice belonging to the original and new genetic backgrounds, and Fig. 2.B depicts the total activity of euploid control mice belonging to the two genetic backgrounds. Univariate tests revealed significant effects of genotype ($F_{1,98} = 20.988$, $*P < 0.001$) and cycle ($F_{1,98} = 71.915$, $*P < 0.001$), and no significant effect of background ($F_{1,98} = 0.031$, $P = 0.861$), on total activity. In addition, we also detected a significant interaction between genotype and cycle ($F_{1,98} = 9.742$, $*P = 0.002$), but no significant interactions between genotype and background ($F_{1,98} = 0.290$, $P = 0.591$) or background and cycle ($F_{1,98} = 0.192$, $P = 0.662$). *Post hoc* analyses revealed that, regardless of genotype and genetic background, mice were more active during the dark cycle than during the light cycle ($*P = 0.008$, 0.010 , < 0.001 , and < 0.001 ; for B6C3 euploid, B6C3.B euploid, B6C3 Ts65Dn, and B6C3.B Ts65Dn, respectively). Additionally, during the light cycle, the mean total activity of control euploid mice did not differ significantly from that of Ts65Dn mice ($P = 0.579$ and $P = 0.378$; for the B6C3 and B6C3.B backgrounds, respectively). During the dark cycle, however, the mean total activity was significantly greater in Ts65Dn mice than in euploid control mice ($*P < 0.001$ for both the B6C3 and B6C3.B backgrounds).

Similar results were found when we analyzed further different components of the animals' locomotor activity. Fig. 3 represents data analyses for the following four components of the 24-h activity: (A) ambulatory activity, (B) rearings, and (C) horizontal and (D) vertical stereotypic activities. Significant genotype- and cycle-dependence were found for all four activity components. In addition, there were significant interactions between genotype and cycle and no background dependence in all four activity components. The results of univariate tests and *post hoc* analyses for each measure are listed in Table 1 and Table 2, respectively.

The new genetic background produces very subtle differences on the Morris water maze performance of Ts65Dn mice and their euploid controls

Using different variations of the Morris water maze task, four independent research groups have generated robust data demonstrating behavioral deficits potentially associated with learning and memory in Ts65Dn mice [17,24,25,26,27,28,29,30,31,32,33,34]. Performance on the hidden platform version of the task is impaired by hippocampal lesion in both rats [35] and mice [36]. However, the stress produced by the forced swimming and the heavy reliance on the animal's motor skills may significantly reduce the *specificity* of the results produced by the

Morris water maze. Although, one might argue that these are exactly the same factors that enhance the *sensitivity* of this behavioral test. Stasko and Costa [17] have made significant strides toward defining potentially confounding features of the water maze deficits seen in Ts65Dn mice. These authors have found, for example, that Ts65Dn mice are significantly more sensitive to potential stressors and more prone to swim-induced hypothermia than euploid control animals. Still, a *mild, but significant* deficit in water maze performance by Ts65Dn mice remains after all these potentially confounding features are factored out. Here, we compared side-by-side the performance of Ts65Dn and control euploid mice of the original background with that of the new B6C3.B-Ts65Dn and B6C3.B euploid mice.

Fig. 4 shows the time taken to reach the platform (latency) as a function of the training block for the hidden, visible, and reverse platform components of the task. We found a significant genotype-dependence for the latency to acquire the hidden platform ($F_{8,456} = 3.367$, $*P < 0.001$, Fig. 4A) and the reverse platform ($F_{8,456} = 7.796$, $*P < 0.001$, Fig. 4C). Genetic background, however, had no significant effect on the acquisition latency for either of these two components of the water maze task ($F_{8,456} = 0.217$, $P = 0.988$, for the hidden platform, and $F_{8,456} = 0.680$, $P = 0.639$, for the reverse platform). In addition, we found no significant interaction between genotype and background for either component of the water maze task ($F_{1,456} = 0.007$, $P = 0.935$, for the hidden platform, and $F_{1,456} = 0.150$, $P = 0.700$, for the reverse platform). For the visible platform component (Fig. 4B), however, neither genotype ($F_{8,456} = 0.681$, $P = 0.565$) nor genetic background ($F_{8,456} = 0.631$, $P = 0.596$) had a significant effect on the latency for acquisition of the task. We also failed to detect any significant interaction between genotype and background for this component of the water maze task ($F_{1,456} = 0.411$, $P = 0.524$). *Post hoc* analyses of the hidden platform data showed significant increases in latency only at the 3rd and 8th trials ($*P = 0.028$ and $*P = 0.042$, respectively) for Ts65Dn of the original background compared to their euploid controls, and only at the 7th and 9th trials ($*P = 0.039$ and $*P = 0.037$) for B6C3.B Ts65Dn and B6C3.B euploid mice. Similar analyses of the reverse platform data revealed more robust differences, with significant increases in latency detected at the 2nd, 4th, 5th, and 6th trials ($*P = 0.038$, $*P = 0.005$, $*P = 0.005$, and $*P < 0.001$, respectively) for standard B6C3 Ts65Dn mice and their euploid controls, and also at the 2nd, 4th, 5th, and 6th trials ($*P = 0.021$, $*P = 0.018$, $*P = 0.009$, and $*P = 0.012$) for B6C3.B Ts65Dn and B6C3.H.B euploid control mice.

Fig. 5B shows dwell times in the trained versus opposite quadrant during the probe trial after the hidden platform training. ANOVA did not reveal either genotype ($F_{1,103} = 0.014$, $P = 0.906$) or background ($F_{1,103} = 0.015$, $P = 0.905$) effects. In addition, no interactions were detected between genotype and background ($F_{1,103} = 0.552$, $P = 0.459$), genotype and platform location ($F_{1,103} = 2.224$, $P = 0.139$), or background and platform location ($F_{1,103} = 0.531$, $P = 0.468$). Still, a significant effect of quadrant location was detected ($F_{1,103} = 20.143$, $*P < 0.001$). In contrast, we detected a significant genotype dependence ($F_{1,52} = 11.735$, $*P = 0.001$), but no significant background dependence ($F_{1,52} = 3.071$, $P = 0.086$), when we analyzed the mean number of times the mice crossed the area where the platform was located during the hidden platform training blocks (Fig. 5C). *Post hoc* analyses demonstrated a significant difference between the performance of Ts65Dn and control euploid mice in the original background ($*P < 0.001$), but the performance difference between B6C3.B Ts65Dn mice and their euploid controls did not reach significance level ($P = 0.161$).

Fig. 5E depicts dwell times in the probe trial after the reverse platform training. Similar to the results for the hidden probe trial, we did not detect any significant genotype ($F_{1,106} < 0.001$, $P = 0.989$) or background ($F_{1,106} = 0.010$, $P = 0.921$) effect. Furthermore, no interactions were detected between genotype and background ($F_{1,106} = 0.008$, $P = 0.927$), or genotype and platform location ($F_{1,106} = 0.000$, $P = 0.995$). However, in this particular experiment, we found a significant interaction between background and platform location ($F_{1,106} = 4.389$, $*P =$

0.039). Again, we detected a significant effect of quadrant location ($F_{1,106} = 18.595$, $*P < 0.001$). In contrast to the hidden platform probe trial, in this component of the task, *post hoc* analyses showed significant differences in mean dwell times in the trained versus opposite quadrant for both control euploid and Ts65Dn mice, but only for animals in the original genetic background ($*P = 0.002$, and $*P < 0.001$, respectively). We failed to detect significant differences in mean dwell times in the trained versus opposite quadrant for B6C3.B euploid control and B6C3.B Ts65Dn mice ($P = 0.265$, and $P = 0.316$, respectively). In addition, for the mean number of trained platform area crossings during the reverse platform probe trial, we found not only a very strong genotype effect ($F_{1,106} = 41.691$, $*P < 0.001$), but also a significant background effect ($F_{1,106} = 4.441$, $*P = 0.040$) (Fig. 5F). *Post hoc* analyses revealed a significant difference in performance between Ts65Dn and control euploid mice of the original B6C3 background ($*P < 0.001$), as well as a significant difference between the performance of B6C3.B Ts65Dn mice and their euploid controls ($*P < 0.001$). In addition, in the reverse platform probe trial, we also detected a significant difference in performance between control euploid mice in the original B6C3 versus B6C3.B control animals ($*P = 0.023$). However, no significant difference was detected between the mean number of platform crossings of Ts65Dn mice in the original B6C3 versus B6C3.B Ts65Dn mice ($P = 0.532$).

Finally, we analyzed the mean dwell time in the pool periphery as a measure of thigmotactic behavior. Figure 6 is a graphic representation of this analysis. In Fig. 6A, we show that during the initial probe trial, naïve mice, regardless of genotype or genetic background, spent approximately 70% of the trial time swimming in the periphery of the pool. Consequently, we did not detect any significant genotype ($F_{1,57} = 0.684$, $P = 0.412$) or background ($F_{1,57} = 0.183$, $P = 0.671$) effect or genotype $\times$ background interaction ($F_{1,57} = 0.368$, $P = 0.546$) during this initial probe trial. During the course of the experiment, however, control euploid mice seem to spend progressively more time in the center area of the pool as part of their search strategy, which did not seem to be the case for Ts65Dn mice, regardless of genetic background. For example, during the probe trial after the hidden platform training (Fig. 6B), the mean percentage of time in the pool periphery was significantly dependent on genotype ($F_{1,52} = 9.990$, $*P = 0.003$), but not significantly dependent on background ($F_{1,52} = 0.702$, $P = 0.406$), and no genotype $\times$ background interaction was detected ($F_{1,52} = 0.148$, $P = 0.703$). *Post hoc* analysis of these data showed that both B6C3 Ts65Dn and B6C3.B Ts65Dn mice spent significantly more time in the periphery than B6C3 and B6C3.B control mice ($*P = 0.046$ and $*P = 0.019$, respectively).

A similar pattern was seen for the probe trial following the reverse platform training (Fig. 6C). In this specific experiment, the mean percentage of time in the pool periphery was significantly dependent on genotype ($F_{1,54} = 11.221$, $*P = 0.001$) and on genetic background ($F_{1,54} = 5.638$, $*P = 0.021$). Although, no genotype $\times$ background interaction was detected ($F_{1,54} = 0.029$, $P = 0.866$). Again, *post hoc* analysis of these data showed that both B6C3 Ts65Dn and B6C3.B Ts65Dn mice spent significantly more time in the periphery than B6C3 and B6C3.B control animals ($*P = 0.014$ and $*P = 0.031$, respectively). However, the same *post hoc* analysis did not detect any significant differences between B6C3 Ts65Dn and B6C3.B Ts65Dn mice ($P = 0.112$) or between B6C3 and B6C3.B euploid control mice ($P = 0.088$).

Contextual and sound-cued fear conditioning in Ts65Dn is impaired in a comparable way in the original and new Ts65Dn stocks

Recently, Costa et al. [37] have shown that Ts65Dn mice display deficits on long-term (24-hour) contextual fear conditioning performance. In the present study, we compared side-by-side the performance of Ts65Dn and control euploid mice of the original background with that of B6C3.B Ts65Dn and B6C3.B euploid mice on a fear conditioning task. Costa et al. [37]

used a protocol involving exclusively contextual fear conditioning. Here, we present data for both contextual and sound cued fear conditioned responses (Figure 7).

Fig. 7A shows the mean percentage of time freezing for Ts65Dn mice in the old and new genetic backgrounds and their respective controls during the 3-minute re-exposure to the training context 24-h post-training. We found a significant genotype-dependence for the percentage of time freezing ($F_{1,43} = 12.7969$, $*P < 0.001$), but no significant background effect ($F_{1,43} = 0.059$, $P = 0.809$) or genotype $\times$ background interaction ($F_{1,43} = 0.080$, $P = 0.779$). In addition, *post hoc* analysis of the data revealed that control euploid mice in both the original B6C3 and the new B6C3.B backgrounds spent a significantly larger percentage of the time freezing than their Ts65Dn counterparts during the context test ($*P = 0.033$ and $*P = 0.006$, respectively).

The results of the auditory pre-stimulus test, in which the animals are simply placed in a completely different box than the one in which they first received the unconditioned stimulus are displayed in Fig. 7B. Data analysis showed no significant genotype ($F_{1,42} = 0.129$, $P = 0.721$) or background ($F_{1,42} = 0.203$, $P = 0.655$) effect. In addition, no genotype $\times$ background interaction was detected ($F_{1,42} = 0.674$, $P = 0.416$). After spending three minutes in this new box, the acoustic-conditioned stimulus was turned on and freezing behavior was quantified for another 3-minute interval. This resulted in the dataset depicted in Fig. 7C. Under this sound-cued protocol, control animals of both genotype spent approximately 70% of the time freezing, whereas the respective Ts65Dn mice spent approximately 50% of the time freezing.

Accordingly, data analysis demonstrated a significant genotype-dependence for the percentage of time freezing ($F_{1,42} = 11.451$, $*P = 0.002$), but no significant background effect ($F_{1,42} = 1.246$, $P = 0.271$) or genotype $\times$ background interaction ($F_{1,42} = 0.010$, $P = 0.922$). Similar to the context test, *post hoc* analysis of the data showed that control euploid mice in both the original B6C3 and the new B6C3.B backgrounds spent a significantly larger percentage of the time freezing than their Ts65Dn counterparts during the sound cued test ($*P = 0.035$ and $*P = 0.011$, respectively).

Robust and comparable decreases in the production of grip forces are seen in the original and new Ts65Dn stocks

In a previous study, Costa et al. [23] have shown that the production of mouse forelimb grip force by Ts65Dn mice was significantly smaller compared to control euploid animals. Here, we compared the forelimb grip strength of Ts65Dn and control euploid mice of the original B6C3 background to the grip strength of B6C3.B Ts65Dn and B6C3.B euploid mice.

Figs. 8A and 8B depict representative digitized traces from the output of the grip strength meter for Ts65Dn and control euploid mice of the original B6C3 background. Figs. 8C and 8D are representative traces from B6C3.B Ts65Dn and B6C3.B euploid mice. These traces illustrate the finding that Ts65Dn mice from both genetic backgrounds produce less grip force than their respective euploid controls and that genetic background does not seem to have a significant effect on the amount of grip force produced. This is confirmed in Fig. 8E, which shows the bar graph representation of the mean grip force values recorded from all four groups of animals. Data analysis revealed a significant genotype-dependence for the measured peak grip force ($F_{1,55} = 35.025$, $*P < 0.001$) and no significant background effect ($F_{1,55} = 0.838$, $P = 0.364$) or genotype $\times$ background interaction ($F_{1,55} = 0.004$, $P = 0.953$). *Post hoc* analysis of the data showed that control euploid mice in both the original B6C3 and the new B6C3.B backgrounds produced significantly larger peak grip forces than their Ts65Dn counterparts ($*P < 0.001$ for both genetic backgrounds). In addition, we also analyzed the mean relative slope of the grip force traces recorded from each of the four groups of mice (Fig. 8F). We found a no significant background dependence ($F_{1,55} = 0.767$, $P = 0.385$) or background $\times$ genotype interaction ($F_{1,55} = 0.160$, $P = 0.691$). Although we found a significant genotype-dependence for this measure ($F_{1,55} = 4.039$, $*P = 0.049$), *post hoc* analysis of the data failed to demonstrate any

significant differences between the mean relative slope of the grip forces produced by control euploid mice in both the original B6C3 and the new B6C3.B backgrounds compared to their Ts65Dn counterparts ($P = 0.263$ and $P = 0.092$, respectively).

Discussion

In this report, we describe the results of several experiments in which we compared side-by-side Ts65Dn mice of a new stock that does not carry the *Pde6b^{rd1}* mutation with mice of the standard Ts65Dn stock. The results can be summarized as follows: (1) the body weight and length of Ts65Dn mice and their euploid controls are not significantly affected by the new genetic background; (2) Ts65Dn in the new genetic background display nocturnal hyperactivity comparable to that seen in the original Ts65Dn stock; (3) the new genetic background may produce very subtle differences on the Morris water maze performance of Ts65Dn mice and their euploid controls; (4) contextual and sound-cued fear conditioning in Ts65Dn is impaired in a comparable way in the original and new Ts65Dn stocks; and (5) robust and comparable decreases in the production of grip forces are seen in the original and new Ts65Dn stocks.

In accordance to previously published data, we found that Ts65Dn mice have lower mean body weight than control euploid animals [23,26,38]. This was true for both Ts65Dn mice of the new stock and for mice of the standard Ts65Dn stock. Surprisingly, this is only the second time comparisons of body length measurements for Ts65Dn mice and control euploid mice have been reported in the literature. Our observations agreed with the previously published data [23], in which the nose to tail base length is shorter in Ts65Dn mice compared to control euploid mice. In the sample of Ts65Dn mice of the new stock used in the present study, the difference between this particular measure in trisomic versus euploid animals did not reach significance. However, when we compared the animal length as measured from nose to the end of the tail, we found similar results for mice of both stocks, i.e., that Ts65Dn mice are significantly shorter than control euploid mice. The reason for this discrepancy is probably because the measurement of the distance between nose and the end of the tail is more objective and less variable, given that it does not depend on arbitrary anatomical landmarks.

The 24-h locomotor activity data for Ts65Dn in both the new genetic and the standard stock is also in agreement with previously published results [26,31]. We found that Ts65Dn mice of the two stocks display nocturnal hyperactivity when compared to control euploid mice. Similar to the previous studies, we analyzed total activity, ambulatory activity, and number of rearings. In addition, we also examined horizontal and vertical stereotypic activities as defined by the number of bursts of stereotypic activity that the animal engages in the horizontal and vertical planes during the 24-h monitoring. Although we recognize the shortcomings of monitoring stereotypic behavior through the use of photocell arrays, our goal was simply to obtain an estimate of stereotypic activity in the horizontal and vertical planes as a means of qualitatively reproducing the finding of spontaneous stereotypy in Ts65Dn mice by Turner et al. [39]. Even with the fairly short “burst terminator time” of 1-s, we found evidence of significantly more stereotypic activity in Ts65Dn mice of both genetic backgrounds in relation to euploid control mice during the dark cycle.

The main goal of the Morris water maze experiments in the present study was to provide a direct comparison of the performance of Ts65Dn of both the new genetic and the standard stock in this ubiquitous behavioral task. However, we also introduced two small changes in the protocol used here when compared to our previous work [17]. First, in the previous study, the water temperature was kept typically at 24 °C (except for a limited number of experiments in which the temperature was reduced purposely to 19 °C). Because, there was still a considerable amount of “floating behavior” when mice swam at 24 °C, we decided to reduce the water temperature to 22 °C, as a compromise between a “comfortable” and an “aversive”

temperature. Interestingly, in our previous work [17], we found no significant genotype difference in the latency of acquisition of the hidden platform at 24 °C, but we found a significant genotype dependence for this measure when the water temperature was maintained at 19 °C. Here, we again found significant genotype dependence, but no background dependence, for this measure at 22 °C. These results emphasize the differential effect of even small changes in the aversiveness of the test conditions on the acquisition of the water maze by Ts65Dn mice versus euploid control mice.

The second change in the protocol used in the present study was the addition of 6 blocks of 4 trials following the visible platform component of the task. This additional experimental step, which was designed to test the flexibility to adapt to new conditions, produced stronger genotype dependence than that seen for the acquisition of the hidden platform task. Again, no background dependence was noted for the latency for acquisition of the reverse platform. However, we noted a few, subtle, background-dependent differences in the performance in the probe trials (see Fig. 5). Of these differences, the only significant background dependent difference we were able to detect was that, in the probe trial following the acquisition of the reverse platform, control euploid mice of the standard background slightly outperformed the control mice of the new background. Furthermore, although we detected small differences statistically, the rank order, i.e., control mice outperforming Ts65Dn mice in absolute terms, was preserved in all experiments, including the probe trials. But, more importantly, the main features of the Morris water maze deficit of Ts65Dn mice in relation to their control euploid siblings were preserved in the new genetic background, including the thigmotactic search pattern they acquire during the water maze training. Indeed, due to this thigmotactic behavior displayed by Ts65Dn mice, it remains unclear whether the observed genotypic differences for many measures in the Morris water maze task (acquisition as well as probe tests) reflect true learning and memory deficits in Ts65Dn mice of either genetic background.

The protocol modifications for the present fear conditioning experiments in relation to one of our previous studies [37] also produced some new and interesting results. In the previous study, we used a protocol involving exclusively contextual fear conditioning. In contrast, here, we present data for both contextual and sound cued fear conditioned responses. We found that Ts65Dn mice (irrespective of their genetic background) display a deficit in these two forms of fear conditioned response. This is of interest because it is widely believed that context fear conditioning in rodents is dependent on both the amygdala and the hippocampus [40,41], whereas sound-cued conditioned fear response is thought to be dependent on the amygdala but not the hippocampus [42]. Over the last decade, however, the concept that the role of the hippocampus in fear conditioning is modality-specific has been challenged by several groups (recently reviewed in [43]). Still, it is clear that, in light of the data presented here, we cannot discount a potential contribution of the amygdala to the deficit in the fear conditioning in Ts65Dn mice. Given that NMDA receptor neurotransmission in the central nucleus of the amygdala has been implicated in the acquisition of conditioned freezing [44], it will be interesting to investigate in a future study whether the NMDA uncompetitive antagonist memantine pharmacologically rescues cued-fear conditioning deficit in Ts65Dn mice in a manner similar to its rescue of contextual fear conditioning deficit in these animals [37].

Finally, in accordance with previously published data by our two research teams [23], we found a significant deficit in the production of grip strength by Ts65Dn mice. Again, we found that this phenotype was not dependent on genetic background. The main reason for choosing this specific assessment of motor function in the present study was the experimental simplicity and the robustness of the Ts65Dn mouse phenotype when assessed by these methods. For example, another robust phenotype representing motor dysfunction that can be seen in Ts65Dn mice involves abnormal gait and gait dynamics. These assessments, however, involve either painstaking paw print analysis by hand [23] or by computerized analysis of digital video images

acquired through high speed systems equipped with specialized software [45]. In contrast, data on the rotarod performance in Ts65Dn mice have been inconsistent among laboratories, with two groups reporting a performance deficit [23,39] and three groups reporting no significant genotype-effect on performance [12,38,46]. Although different rotarod protocols have been used sometimes by these different groups, in one occasion the same protocol has been used, but it yielded different results in different publications involving overlapping authors [38,39]. One potential source for these discrepancies, might again reside in genotype-dependent differences in response to stress induced by repeated handling during batteries of behavioral assessments, but experiments to address this issue are beyond the scope of the present study.

In summary, we found that, except for very subtle differences in water maze performance, there were no significant differences between Ts65Dn mice of the two backgrounds in the measures assessed in the present study. Future studies perhaps involving electrophysiological and histological techniques to assess the phenotype of Ts65Dn mice of the new stock might be in order to validate fully this new stock. However, given how genetically close the new stock is to the old stock, and the results presented here, we anticipate that the new stock will soon replace the old one as the main stock of Ts65Dn mouse available for research. This would be a particularly welcome development, given the ever increasing use of Ts65Dn mice in whole animal pharmacological rescuing studies aimed at testing pre-clinically potential therapeutic agents targeted at ameliorating some modalities of cognitive deficits or neuropathologies associated with DS [31,33,34,37,46,47,48,49,50,51]. Pharmacological experiments, however, are notorious for requiring large numbers of animals. Given the low reproductive performance of Ts65Dn mice, coupled to the presence of the retinal degeneration mutation *Pdeb^{rdl}* in the standard Ts65Dn stock, these efforts have been limited in scope. The elimination of the retinal degeneration mutation *Pdeb^{rdl}* from the Ts65Dn stock has achieved two practical goals without producing any noteworthy phenotypic alterations: (1) it allows for an increase in the generation of animals that can be used in behavioral assessment from breeding colonies by about 25%; (2) it decreases colony maintenance costs by obviating the need for retinal generation screening. This potential increase in the availability of Ts65Dn mice should result in a more widespread use of the animals in translational efforts in DS research.

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Fig. 1.

Mean body weight and length of Ts65Dn mice versus euploid control mice. (A) represents mean body weights of euploid control (n=15) and Ts65Dn (n=15) mice of the standard genetic background as well as euploid control (n=16) and Ts65Dn (n=16) mice of the new background. (B) and (C) show mean body length for these same animals, as measured from nose to the base of the tail and nose to the tip of tail, respectively. The bars represent the mean $\pm$ SEM. Due to space considerations, the designations “Control” and “Ts65Dn” have been used in all figures to represent euploid control and Ts65Dn mice of the standard genetic background (B6C3) and the nonstandard abbreviations “Control.B” and “Ts65Dn.B” have been used to denote euploid control and Ts65Dn mice of the new background (B6C3.B).

Fig. 2.

Patterns of 24-h spontaneous locomotion in Ts65Dn mice and euploid control mice of the two genetic backgrounds. (A) depicts mean total activity in one hour as a function of time for a group of Ts65Dn mice in the B6C3 background ($n=15$; filled squares) compared to a group of Ts65Dn mice in the B6C3.B background ($n=12$; filled inverted triangles). (B) represents mean total activity in one hour as a function of time for a group of B6C3 control euploid mice ($n=15$; open squares) compared to the control euploid mice in the B6C3.B background ($n=11$; open inverted triangles). In this figure, time = 0 means the beginning of the “dark cycle” (i.e. lights off at 7:00 PM). For each graph, symbols represent the mean $\pm$ SEM.

Fig. 3. Different components of the animals' locomotor activity. (A) represents ambulatory activity, (B) rearings, (C) horizontal stereotypic activity, and (D) vertical stereotypic activity. The numbers of animals in each group (genotype) are the same as those given in Fig. 2. In each graph, the bars represent the mean $\pm$ SEM.

Fig. 4.

Morris water-maze results from euploid control mice of the standard background (B6C3-control group; open squares; n=16), euploid control mice of the new background (B6C3.B; open inverted triangles; n=14), B6C3-Ts65Dn mice (filled squares; n=16), and B6C3.B-Ts65Dn mice (filled inverted triangles; n=15) across blocks of training. (A) illustrates escape latencies for the hidden platform task. (B) represents escape latencies for the visible platform task. (C) depicts escape latencies for the reverse platform task. The symbol * was used to denote statistically significant differences ($p < 0.05$) in mean latency between the B6C3-Ts65Dn group and its euploid control group in specific blocks of trials. The symbols † and †† were used to denote statistically significant differences in mean latency between the B6C3.B-

Ts65Dn group and its euploid control group in specific blocks of trials ($p < 0.05$ and $p < 0.01$, respectively). In each graph, symbols represent the mean $\pm$ SEM.

Figure 5.

Morris water-maze probe trials results from B6C3-euploid control, B6C3-Ts65Dn, B6C3.B-euploid control, and B6C3.B-Ts65Dn mice. (A) and (D) depict the relative locations of the trained versus the opposite quadrants in the hidden (B6C3-control n=15; B6C3-Ts65Dn n=15; B6C3.B-control n=12; B6C3.B-Ts65Dn n=14) and reverse (B6C3-control n=14; B6C3-Ts65Dn n=16; B6C3.B-control n=13; B6C3.B-Ts65Dn n=15) probe trials. (B) and (F) represent the mean search times in trained versus the opposite quadrants for each genotype/genetic background. (C) and (G) show the number of platform-region crossings during the probe trials for each genotype/genetic background. For each graph, the bars represent the mean $\pm$ SEM.

Figure 6.

Morris water-maze mean dwell times in the periphery during probe trials as a measure of thigmotactic behavior for B6C3-euploid control, B6C3-Ts65Dn, B6C3.B-euploid control, and B6C3.B-Ts65Dn mice. (A), (B), and (C) depict results from initial, hidden, and reverse probe trials, respectively. In each graph, the bars represent the mean $\pm$ SEM.

Figure 7.

Mean percentage of time freezing for B6C3-euploid control, B6C3.B-euploid control, B6C3-Ts65Dn mice, and B6C3.B-Ts65Dn mice during the 3-minute re-exposure to the training context/cue 24-h post-training. (A), (B), and (C) depict results for context, neutral environment, and neutral environment plus auditory cue, respectively. In each graph, the bars represent the mean $\pm$ SEM.

Figure 8.

Grip force measurements for Ts65Dn mice in the old and new genetic backgrounds and their respective controls. (A), (B), (C), and (D) show representative examples of grip force recordings from B6C3-euploid control, B6C3-Ts65Dn, B6C3.B-euploid control, and B6C3.B-Ts65Dn mice, respectively. (E) represents the mean peak force results from B6C3-euploid control (n=14), B6C3-Ts65Dn (n=15), B6C3.B-euploid control (n=14), and B6C3.B-Ts65Dn (n=16) mice. (E) shows the mean relative slopes for the grip force curves obtained from the four groups of animals. In the graphs depicted in (E) and (F), the bars represent the mean $\pm$ SEM.

Table 1

ANOVA univariate test results for the four activity components: (1) ambulatory activity, (2) number of rearings, (3) horizontal stereotypic activity, and (4) vertical stereotypic activity. These components were analyzed in terms of their dependence on three factors: (1) genotype (Ts65Dn $\times$ euploid control), (2) light cycle (light $\times$ dark), (3) genetic background (B6C3 $\times$ B6C3.B). In addition, we analyzed the interaction between genetic background and light cycle.

Activity Component	Univariate tests (F1,98, P)					
	Genotype	Light Cycle	Background	Genotype $\times$ Background	Genotype $\times$ Light Cycle	Background $\times$ Light Cycle
Ambulatory activity	12.822, <0.001	0.143, 0.706	78.595, <0.001	7.838, 0.006	0.478, 0.490	0.112, 0.739
Rearings	19.309, <0.001	0.314, 0.576	108.560, <0.001	7.793, 0.006	0.024, 0.877	0.126, 0.723
Horizontal stereotypic activity	30.669, <0.001	0.639, 0.426	126.579, <0.001	6.276, 0.014	0.068, 0.795	0.424, 0.517
Vertical stereotypic activity	21.635, <0.001	0.3295, 0.567	102.864, <0.001	9.481, 0.003	0.016, 0.899	0.101, 0.752

Table 2

PLSD *post hoc* analysis results for the genotype and light cycle dependence of the four activity components: (1) ambulatory activity, (2) number of rearings, (3) horizontal stereotypic activity, and (4) vertical stereotypic activity.

Activity Component	Activity Components - <i>Post hoc</i> Analyses (P values)							
	Genotype Effect (Control vs. Ts65Dn)				Light Cycle Effect (Dark vs. Light)			
	B6C3H	B6C3H.B	B6C3H.B	B6C3H.B	B6C3H	B6C3H.B	B6C3H.B	B6C3H.B
	Dark	Light	Dark	Light	Control	Ts65Dn	Control	Ts65Dn
Ambulatory activity	*0.0036	0.9053	*0.0010	0.5304	*0.0022	*< 0.0001	*0.0049	*< 0.0001
Rearings	*0.0002	0.3380	*0.0013	0.5087	*< 0.0001	*< 0.0001	*0.0012	*< 0.0001
Horizontal stereotypic activity	*< 0.0001	0.1537	*0.0002	0.1147	*< 0.0001	*< 0.0001	*< 0.0001	*< 0.0001
Vertical stereotypic activity	*< 0.0001	0.3305	*0.0005	0.5357	*0.0001	*< 0.0001	*0.0030	*< 0.0001