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Location- and sex-specific differences in weight and motor coordination in two commonly used mouse strains

SUBJECT AREAS:

ANIMAL DISEASE
MODELS

NEURODEGENERATIVE DISEASES

NEURODEVELOPMENTAL
DISORDERS

METABOLIC DISORDERS

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Several studies have shown that environmental factors can affect the outcome of behavioral experiments, shedding doubts on the inter-laboratory reproducibility of behavioral test results. When our laboratory moved from the University of Rochester, Rochester, NY, to Sanford Research in Sioux Falls, SD, our mouse colony was also transferred and the new environment caused strain-dependent changes in the weight, motor coordination and motor learning capability of mice. Here we report the observed changes for two wild type mouse strains commonly used in transgenic studies, C57BL/6J and 129S6/SvEv, and show that the type of rodent diet is partially responsible for the geographical location-specific differences. We also found sex-specific differences in weight and motor coordination in both mouse strains. Our results show that environmental factors specific to a geographical location can change the body weight, motor coordination and motor learning capability of wild type mice commonly used as controls in transgenic studies.

Transgenic mouse models of human neurological and neurodegenerative diseases undergo extensive behavioral phenotyping. The observed phenotypes, their severity and age of onset, however, can vary from lab to lab. It has been shown that lab-to-lab variations in environmental factors strongly influence the outcome of behavioral studies¹⁻³. The best example is the study in which three laboratories at different geographical locations, one in Albany, NY, another in Portland, Oregon, and a third in Edmonton, Canada, tested the same seven mouse strains in six behavioral tests using the same apparatus, same protocols, and equating many environmental variables¹. Though some results were similar, most of the behavioral test results were quite different depending on the lab where the test was performed¹. Later studies determined that seemingly insignificant factors such as the experimenter (differences in animal handling)^{4,5}, the cage type [microisolator (high ventilation) vs. filter-top (low ventilation)]⁶, the cage density^{4,7}, the presence of cage enrichment^{7,8}, the form of cage enrichment (cardboard roll vs. mouse house)⁹, and even the cage position on the rack in the vivarium¹⁰ can significantly alter rodent behavior. Environmental variables can also modify neuroanatomy⁶ and can even change the phenotypes of transgenic mice¹¹. A study compared C57BL/6 mice kept either in microisolator (high ventilation) or filter-top (low ventilation) cages, and found that mice kept in microisolator cages were significantly more aggressive and the cellular structure of their olfactory bulb markedly changed⁶. Cudilo *et al.* (2007) demonstrated that the arterial pathologies manifested in fibulin-4^{+/-} mice can be reduced by cage enrichment (adding a tunnel and a wheel in the cage)¹¹.

When our lab, together with our mouse colony, moved from Rochester, New York, to Sioux Falls, South Dakota, we tried to reproduce our previous behavioral test results obtained in Rochester, NY. We had to realize that the weight and motor coordination of both control wild type and disease model transgenic mice changed in Sioux Falls, SD. Here we describe the observed changes for two wild type mouse strains commonly used in transgenic studies, C57BL/6J and 129S6/SvEv, and show that the type of rodent diet was partially responsible for the geographical location-specific differences. In both mouse strains, we also found sex-specific differences in weight and motor coordination.

Our results demonstrate that environmental factors specific to a geographical location can change the body weight, motor coordination and motor learning capability of wild type mice commonly used as controls in transgenic studies.

Results

Geographical location-specific differences in the weight and motor coordination of C57BL/6J and 129S6/SvEv mice. When our lab, together with our mouse colony, moved from Rochester, New York, to Sioux Falls,



South Dakota, we tried to reproduce our previous rotarod test results obtained in Rochester, NY. The behavioral testing started 6 months after our mouse colony had arrived in Sioux Falls. Surprisingly, the previously observed, pronounced difference in rotarod performance between our control wild type (WT) and disease model transgenic mice was largely diminished in Sioux Falls. Since the sensitivity of the rotarod test depends on the task parameters, particularly on the acceleration^{12–14}, we compared the motor coordination of WT and disease model mice using different rotarod protocols and accelerations. During these experiments we realized that the weight and motor coordination of wild type C57BL/6J and 129S6/SvEv mice changed in Sioux Falls, SD. These two strains are widely used to generate transgenic mice, and serve as wild type controls in transgenic studies.

One-month-old C57BL/6J mice, both males and females, are remarkably heavier in Sioux Falls, SD, than they were in Rochester, NY (Fig. 1a; $p < 0.001$ for males and $p = 0.009$ for females, 2-way ANOVA with Bonferroni's post-test). At the age of 5 months, C57BL/6J male mice are 4.7 g heavier in Sioux Falls than they were in Rochester (Fig. 1a; $p = 0.0001$, unpaired t-test), and their motor coordination is severely impaired in Sioux Falls (Fig. 1b, $p < 0.0001$, unpaired t-test). We only tested 5-month-old C57BL/6J males in Rochester since our C57BL/6J females were used as breeders until the age of 6–7 months.

In contrast to C57BL/6J mice, 1-month-old 129S6/SvEv mice, both males and females, became significantly lighter in Sioux Falls,

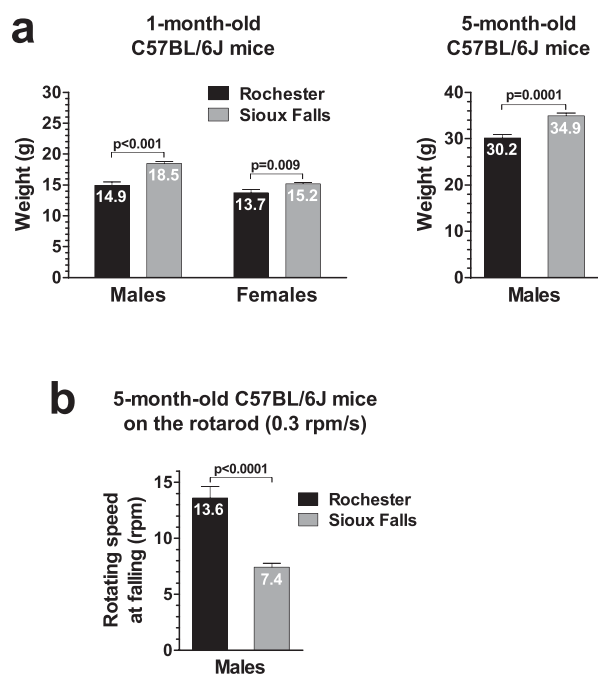


Figure 1 | Location-specific differences in the weight and motor coordination of C57BL/6J mice. (a) C57BL/6J mice are heavier in Sioux Falls, SD, than they were in Rochester, NY. Weight of mice at the age of 1 month (left graph, Sioux Falls: $n = 13$ – 14 ; Rochester: $n = 5$) and 5 months (right graph, Sioux Falls: $n = 24$; Rochester: $n = 12$). Columns and bars represent mean \pm S.E.M of weight (g). Two-way ANOVA with Bonferroni's post-test was applied to compare the weight of 1-month-old males and females, respectively, in Rochester and Sioux Falls; unpaired t-test was used to compare the weight of 5-month-old male mice. (b) Rotarod performance of 5-month-old male C57BL/6J mice in Sioux Falls, SD ($n = 24$), and Rochester, NY ($n = 12$). An accelerating rotarod was used to measure the motor coordination of mice. The rotarod accelerated from 0 rpm at 0.3 rpm/s. Columns and bars represent mean \pm S.E.M of the rotating speed (rpm) when mice fell from the rotating rod. Unpaired t-test was used to compare the rotarod test results.

SD, than they were in Rochester, NY (Fig. 2a; $p = 0.005$ for males and $p < 0.0001$ for females, 2-way ANOVA with Bonferroni's post-test). The motor learning capability of 129S6/SvEv mice dramatically changed in Sioux Falls. One-month-old 129S6/SvEv mice in Rochester were able to learn in a 1st rotarod test and significantly improved their motor coordination in a 2nd rotarod test (Fig. 2b; $p = 0.024$ for males and $p = 0.001$ for females, repeated measures 2-way ANOVA with Bonferroni's post-test). One-month-old 129S6/SvEv mice in Sioux Falls, either males or females, did not improve at all in the 2nd rotarod test (Fig. 2b).

Diet is partially responsible for the location-specific differences.

All the above results raised the question: why are the mice different in Sioux Falls? The significant weight differences pointed to the diet. Indeed, the vivarium at the University of Rochester in Rochester, NY, and the vivarium at Sanford Research in Sioux Falls, SD, used different types of rodent diets. In Rochester, mice received the LabDiet 5010 chow (LabDiet, Richmond, IN), whereas in Sioux Falls, breeders received the Teklad Global 2019 diet (Harlan Laboratories, Indianapolis, IN) and non-breeders (after weaning on postnatal day 21) were kept on the Teklad Global 2018 diet (Harlan Laboratories, Indianapolis, IN). Because of the notable

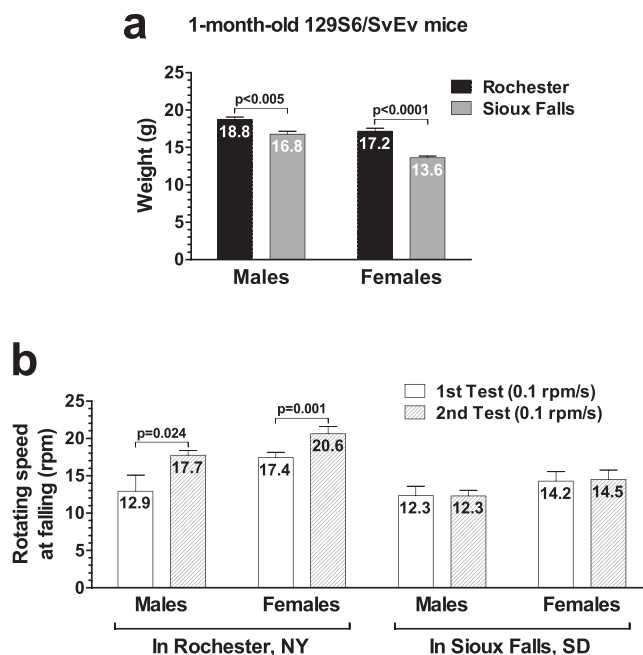


Figure 2 | Location-specific differences in the weight and motor learning of 1-month-old 129S6/SvEv mice. (a) One-month-old 129S6/SvEv mice are lighter in Sioux Falls, SD, than they were in Rochester, NY. (Sioux Falls: 34 males and 29 females; Rochester: 8 males and 19 females). Columns and bars represent mean \pm S.E.M of weight (g). Two-way ANOVA with Bonferroni's post-test was applied to compare the weight of males and females, respectively, in Rochester and Sioux Falls (b) Motor learning capability of 1-month-old 129S6/SvEv mice in Sioux Falls, SD, and Rochester, NY. An accelerating rotarod was used to measure the motor coordination and motor learning of mice (Sioux Falls: 8 males and 8 females; Rochester: 3 males and 16 females). The rotarod accelerated from 0 rpm at 0.1 rpm/s. Columns and bars represent mean \pm S.E.M of the rotating speed (rpm) when mice fell from the rotating rod. Between the 1st and 2nd rotarod tests mice had a 3-h rest interval. Mice in Rochester, NY, effectively learned in the 1st rotarod test and their motor coordination significantly improved in the 2nd rotarod test. Mice in Sioux Falls, either males or females, did not improve at all in the 2nd rotarod test. Repeated measures 2-way ANOVA with Bonferroni's post-test was used to compare the 1st and 2nd rotarod test results of the same mice.



differences in protein, vitamin and fat contents between the rodent diets in Rochester and Sioux Falls (Table 1), we assumed that the diet change was the primary cause of the observed alterations in weight and motor skills. Therefore, a group of 129S6/SvEv mice were kept on LabDiet 5010 (“Rochester diet”) for four generations in Sioux Falls, and they were compared to mice exclusively fed with the Teklad Global 2018 diet (“Sioux Falls diet”) for four generations. One-month-old males on the 5010 diet became markedly heavier than males on the 2018 diet (Fig. 3a; $p < 0.001$, 2-way ANOVA with Bonferroni’s post-test), whereas the weights of females on the 5010 and 2018 diets were similar (Fig. 3a). Comparing the weight of 1-month-old 129S6/SvEv mice fed with the 5010 diet in Rochester and Sioux Falls, no difference between males was found (Fig. 3b). Females, however, were significantly lighter in Sioux Falls than in Rochester (Fig. 3b; $p = 0.001$, 2-way ANOVA with Bonferroni’s post-test).

One-month-old 129S6/SvEv mice in Rochester, NY, (which were kept on the 5010 diet) were able to learn in a 1st rotarod test and significantly improved their motor coordination in a 2nd rotarod test (Fig. 2b). The motor learning capability of 129S6/SvEv mice, however, was dramatically impaired in Sioux Falls, SD (Fig. 2b). To examine if keeping the mice exclusively on the 5010 or 2018 diet in Sioux Falls restored motor learning we used two consecutive rotarod tests. Surprisingly, only males kept on the 5010 diet and females kept on the 2018 diet displayed effective motor learning and significantly improved their motor coordination in the 2nd rotarod test (Fig. 3c; * $p = 0.022$ for males on the 5010 diet and # $p = 0.016$ for females on the 2018 diet, repeated measures 2-way ANOVA with Bonferroni’s post-test). No statistically significant difference in motor coordination, either in the 1st or 2nd rotarod test, was found between males or females kept on the two different diets (Fig. 3c, two-way ANOVA with Bonferroni’s post-test).

Sex-specific differences in the weight and motor coordination of 129S6/SvEv and C57BL/6J mice. Independently of the geographical location and diet, 1-month-old 129S6/SvEv females were always significantly lighter than males (Fig. 2a: $p = 0.0229$ in Rochester and Fig. 4: $p = 6.28 \times 10^{-9}$ in Sioux Falls, unpaired t-test; Fig. 3a; $p < 0.0001$ on the 5010 diet and $p < 0.001$ on the 2018 diet, 2-way ANOVA with Bonferroni’s post-test). However, the motor coordination of males and females, as measured by the rotarod test, was similar (Fig. 2b, Fig. 3c and Fig. 4).

In Rochester, NY, there was no difference in weight between 1-month-old C57BL/6J males and females (Fig. 1a, $p = 0.16$, unpaired t-test). In Sioux Falls, 1-month-old C57BL/6J male mice were markedly heavier than females (Fig. 5a, $p = 2.25 \times 10^{-9}$, unpaired t-test), but the motor coordination of males and females, as measured by the rotarod test, was similar (Fig. 5a). At the age of 5 months, C57BL/6J males in Sioux Falls were 10.3 g heavier than females (Fig. 5b; $p = 8.64 \times 10^{-12}$, unpaired t-test). The motor coordination

of 5-month-old C57BL/6J males and females was compared in two different rotarod tests separated by a 3-h rest interval. In both rotarod tests, males fell from the rotarod at significantly lower rotating speed than females (Fig. 5b; $p < 0.001$ in the 1st test and $p < 0.0001$ in the second test, 2-way ANOVA with Bonferroni’s post-test).

Discussion

When our lab together with our mouse colony moved from Rochester, NY, to Sioux Falls, SD, we observed geographical location-specific differences in the weight and motor coordination of C57BL/6J and 129S6/SvEv mice, two wild type mouse strains commonly used in transgenic studies. To our knowledge this is the first report on behavioral testing of the same mouse colony by the same research group at two quite different geographical locations, comparing males and females. Several previous studies have shown that changes in environmental factors can significantly affect the outcome of behavioral studies. The experimenter (differences in animal handling)^{4,5}, the cage type [microisolator (high ventilation) vs. filter-top (low ventilation)]⁶, the cage density^{4,7}, the presence of cage enrichment^{7,8}, the form of cage enrichment (cardboard roll vs. mouse house)⁹, and the cage position on the rack in the vivarium¹⁰ all have been shown to significantly alter rodent behavior. Environmental variables can also modify neuroanatomy⁶ and can even change the phenotypes of transgenic mice¹¹.

After our lab and mouse colony moved from Rochester, NY, to Sioux Falls, SD, several environmental factors remained identical. In both Rochester and Sioux Falls, our mice were housed in microisolator cages (4 mice/cage), had 100% corn cob bedding, and the same person performed the rotarod testing. Another important environmental factor is the diet. Alterations in the composition and/or components of the rodent diet can have a major effect on neurobehavior^{15,16}. It turned out that our mice were on a different diet in Rochester, NY, than in Sioux Falls, SD, and there were considerable differences between the Rochester and Sioux Falls diets in protein, fat and vitamin contents. Both the breeding (2019) and non-breeding (2018) Teklad Global diets in Sioux Falls contained a markedly lower vitamin B₁ level than the LabDiet 5010 in Rochester. Since vitamin B₁ is essential to have appetite, the difference in vitamin B₁ content alone could explain why 129S6/SvEv mice are lighter in Sioux Falls than they were in Rochester, NY. Indeed, when one-month-old 129S6/SvEv males were kept on the 5010 diet in Sioux Falls, they reached similar weight than in Rochester, NY (Fig. 3b). 129S6/SvEv females kept on the 5010 diet in Sioux Falls, however, remained markedly lighter than they were in Rochester on the 5010 diet (Fig. 3b), indicating that environmental factors other than the diet have a major effect on the weight of 129S6/SvEv females.

In contrast to 129S6/SvEv mice, C57BL/6J mice became heavier in Sioux Falls (Fig. 1). While the lower vitamin B₁ content of the Sioux Falls diets (2018 and 2019) probably decreased the appetite of

Table 1 | Rodent diets used in Rochester, NY, and Sioux Falls, SD, differ in protein, vitamin and fat contents. LabDiet 5010 was autoclaved in Rochester, NY. Teklad Global 2018 and 2019 are non-autoclavable diets

	Rochester, NY	Sioux Falls	Sioux Falls
	Diet for breeders and non-breeders	Diet for non-breeders (after weaning)	Diet for breeders and mothers with pups
	LabDiet 5010 (autoclavable)	Teklad Global 2018	Teklad Global 2019
Protein	24.6%	18%	19%
Vitamin E (IU/kg)	61	110	110
Vitamin K ₃ (mg/kg)	3.4	50	50
Vitamin B ₁ (mg/kg)	91 (~27 after autoclaving)	17	17
Pantothenic acid (mg/kg)	26	33	33
Choline (mg/kg)	2200	1200	1200
Fat (by ether extraction)	4.8%	6.2%	-
Fat (by acid hydrolysis)	5.5%	-	9%

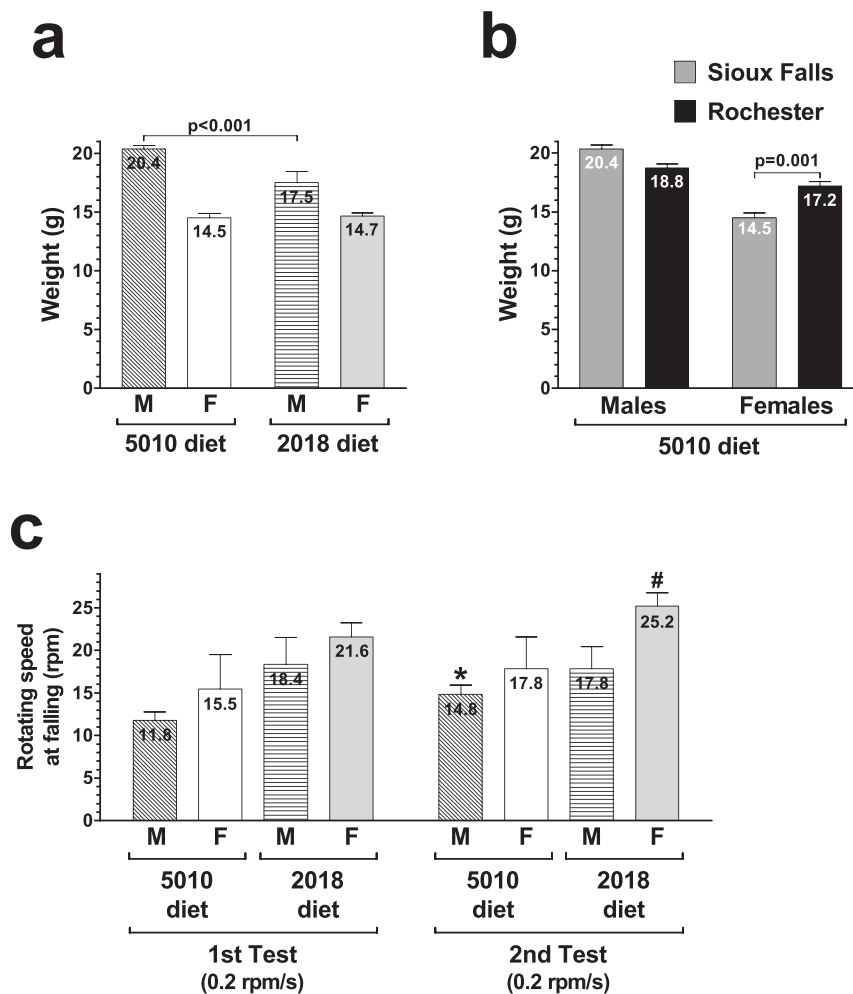


Figure 3 | Diet is partially responsible for the location-specific differences in the weight and motor learning capability of 1-month-old 129S6/SvEv. A group of 129S6/SvEv mice were kept on LabDiet 5010 (“Rochester diet”) for four generations in Sioux Falls, and they were compared to mice exclusively fed with the Teklad Global 2018 diet (“Sioux Falls diet”) for four generations. (a) Weight of 1-month-old 129S6/SvEv male (M) and female (F) mice on the two different rodent diets ($n = 4-8$). Males on the 5010 diet became markedly heavier than males on the 2018 diet ($p < 0.001$, 2-way ANOVA with Bonferroni’s post-test). Columns and bars represent mean \pm S.E.M of weight (g). (b) Weight of 1-month-old 129S6/SvEv male and female mice kept on the LabDiet 5010 diet in Sioux Falls, SD, and Rochester, NY., respectively (Rochester: 8 males and 19 females; Sioux Falls: 5 males and 4 females). Columns and bars represent mean \pm S.E.M of weight (g). The weight of males is similar, but females are significantly lighter in Sioux Falls than they were in Rochester ($p = 0.001$, 2-way ANOVA with Bonferroni’s post-test). (c) Rotarod performance of 1-month-old 129S6/SvEv mice kept either on the 5010 (“Rochester”) diet or on the 2018 (“Sioux Falls”) diet in Sioux Falls ($n = 4-8$). Males kept on the 5010 diet and females kept on the 2018 diet displayed effective motor learning and significantly improved their motor coordination in the 2nd rotarod test (* $p = 0.022$ for males on the 5010 diet and # $p = 0.016$ for females on the 2018 diet). An accelerating rotarod was used to measure the motor coordination and motor learning of mice. The rotarod accelerated from 0 rpm at 0.2 rpm/s. Columns and bars represent mean \pm S.E.M of the rotating speed (rpm) when mice fell from the rotating rod. Between the 1st and 2nd rotarod tests mice had a 3-h rest interval. Repeated measures 2-way ANOVA with Bonferroni’s post-test was applied to compare the 1st and 2nd rotarod test results of the same mice: * $p = 0.022$, # $p = 0.016$.

C57BL/6J mice, as well, the higher fat content of the 2018 and 2019 diets resulted in an overall weight gain due to the susceptibility of C57BL/6J mice to diet-induced obesity¹⁷.

Correlation analysis within our male and female 129S6/SvEv and C57BL/6J groups revealed no statistically significant correlation between the body weight and rotarod performance. A similar lack of correlation has been reported for 129S6/SvEv mice¹⁸. McFadyen *et al.* (2003), however, found a significant negative correlation between the body weight and rotarod performance in 2–3-month-old C57BL/6J mice¹⁹. We only tested 1- and 5-month-old C57BL/6J mice, and the age difference may be responsible for the distinct findings.

Besides the weight, the motor learning capability of 129S6/SvEv mice also changed in Sioux Falls. Unlike 129S6/SvEv mice kept on the 5010 diet in Rochester, NY, 129S6/SvEv males and females on the Sioux Falls diets [2019 diet until weaning (P21), 2018 diet afterward]

were not able to improve their rotarod performance in a second rotarod test (Fig. 2b). When 129S6/SvEv mice were kept either on the 5010 or the 2018 diet for several generations in Sioux Falls, males on the 5010 diet and females on the 2018 diet regained their motor learning capability (Fig. 3c). This change cannot be related to the weight at least in the case of females, since their weight was the same on the 5010 and 2018 diets (Fig. 3a).

Investigating other environmental factors that can potentially affect the weight and motor coordination of our mice, we found a marked difference in the drinking water quality between Rochester and Sioux Falls. While mice received reverse osmosis-purified water in Rochester, NY, autoclaved tap water was their drinking water in Sioux Falls, SD. The tap water in Sioux Falls contains relatively high concentrations of CaCO_3 (267 mg/l) and sulphate (227 mg/l). The significant difference in the chemical components of the drinking

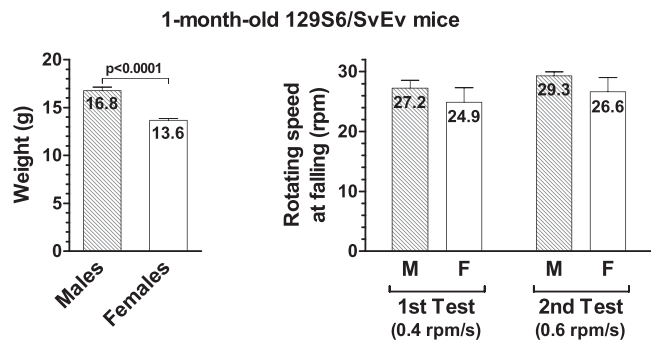


Figure 4 | Sex-specific differences in the weight but not in motor coordination of 1-month-old 129S6/SvEv mice in Sioux Falls, SD.

Breeders received the Teklad Global 2019 diet and non-breeders (after weaning on postnatal day 21) were kept on the Teklad Global 2018 diet. (Left graph) One-month-old 129S6/SvEv males ($n = 34$) are significantly heavier than females ($n = 29$). Columns and bars represent mean \pm S.E.M of weight (g). Unpaired t-test was used to compare the weight of males and females. (Right graph) The rotarod performance of 1-month-old 129S6/SvEv males ($n = 9$) and females ($n = 10$) is similar. An accelerating rotarod was used to measure the motor coordination of mice. Mice were tested in two consecutive rotarod tests (1st: from 0 rpm at 0.4 rpm/s, 2nd: from 0 rpm at 0.6 rpm/s) separated by a 20-min rest interval. Columns and bars represent mean \pm S.E.M of the rotating speed (rpm) when mice fell from the rotating rod. Two-way ANOVA with Bonferroni's post-test was applied to compare the rotarod performance of 1-month-old male and female mice.

water for mice in Sioux Falls and Rochester could contribute to the phenotypic changes we observed in our mouse colony. An additional notable environmental difference was that the nesting material was compressed cotton fibers (Nestlet, Ancare, Bellmore, NY) in Rochester, and paper (TEK-Fresh Cellulose, Harlan Laboratories, Indianapolis, IN) in Sioux Falls. Further variations were that different persons managed and took care of our mouse colony in Sioux Falls than in Rochester, and cage changing was more frequent in Sioux Falls (twice a week) than in Rochester (once every two weeks). These differences might also have a role in the phenotypic changes of our mice.

When our mice were tested in two consecutive rotarod tests on the same day, one could assume that fatigue might have played a role in the second test, causing a poor motor performance. This was not the case, however: mice in the more challenging 2nd test performed similarly or even better than in the first test (see Figs. 2b, 3c, 4 and 5b). Furthermore, we have previously demonstrated, by measuring muscle strength, blood pH and blood levels of partial CO₂, lactate and glucose, that even our longest rotarod protocol (2 consecutive rotarod tests with a 0.1 rpm/s acceleration and 240 s cut-off time, when mice are able to stay on the rotating rod for relatively long times, see Fig. 2b) does not result in fatigue²⁰.

Many human diseases exhibit sex differences in age of onset, disease progression or disease severity²¹. Despite this fact, female rodents are rarely used in behavioral studies, due to the concern of the potential variability related to the estrous cycle. A recent study, however, demonstrated that the behavior of C57BL/6J mice (in contrast to BALB/cByJ mice) remained stable across all four phases of the estrous cycle in all the tests utilized including open field, rotarod, startle reflex and pre-pulse inhibition, tail flick and hot plate²². The sex-specific differences in weight, motor coordination and motor learning we found in our present study also stress the necessity to test both males and females when phenotyping mouse models of human diseases. Currently we keep our mouse colony exclusively on the Teklad Global 2018 diet, and studies are in progress to compare our transgenic disease models on the 129S6/SvEv and C57BL/6J backgrounds, males and females separately in different behavioral tests in order to identify the best mouse model for therapeutic studies.

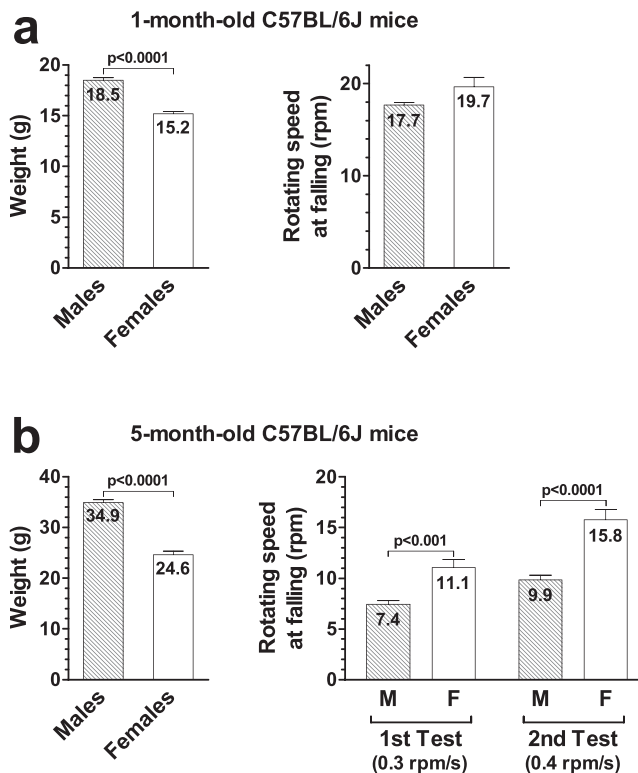


Figure 5 | Sex-specific differences in the weight and motor coordination of C57BL/6J mice in Sioux Falls, SD.

Breeders received the Teklad Global 2019 diet and non-breeders (after weaning on postnatal day 21) were kept on the Teklad Global 2018 diet. (a) A significant difference in the weight but not rotarod performance of 1-month-old C57BL/6J males and females. (Left graph) One-month-old C57BL/6J males ($n = 13$) are significantly heavier than females ($n = 14$). Columns and bars represent mean \pm S.E.M of weight (g). Unpaired t-test was used to compare the weight of males and females. (Right graph) The rotarod performance of 1-month-old C57BL/6J males ($n = 13$) and females ($n = 14$) is similar. An accelerating rotarod was used to measure the motor coordination of mice. The rotarod accelerated from 0 rpm at 0.4 rpm/s. Columns and bars represent mean \pm S.E.M of the rotating speed (rpm) when mice fell from the rotating rod. Unpaired t-test was used to compare the rotarod test results. (b) Five-month-old C57BL/6J males are markedly heavier than females (left graph), and fell from the rotarod at significantly lower rotating speed (right graph); 24 males and 11 females. Columns and bars represent mean \pm S.E.M of weight (g; left graph) and of the rotating speed (rpm) when mice fell from the rotating rod (right graph). Between the 1st (from 0 rpm at 0.3 rpm/s) and 2nd (from 0 rpm at 0.4 rpm/s) rotarod tests mice had a 3-h rest interval. Unpaired t-test was used to compare the weight of 5-month-old males and females, and 2-way ANOVA with Bonferroni's post-test was applied to compare the rotarod test results of 5-month-old males and females.

In summary, we showed that environmental factors specific to a geographical location can change the body weight, motor skills and motor learning capability of wild type mice. Our study warns the research community that mouse behavioral phenotypes determined by a unique combination of environmental and genetic factors can be specific to a geographical location or to a research institute. Our results also emphasize the importance of reporting the type of rodent diet used in a study to improve inter-laboratory reproducibility of behavioral test results.

Methods

Animals. In this study 129S6/SvEv and C57BL/6J wild type mice maintained in our mouse colony were used. In both Sioux Falls, SD, and Rochester, NY, all of our mice were housed in individually vented microisolator cages (4 mice/cage) with *ad libitum*



access to food and water. In Rochester, NY, corn cob bedding (Bed-o-Cobs, Andersons Lab Bedding Products, Maumee, OH) and cotton fiber enrichment (Nestlet, Ancare, Bellmore, NY) were used. In Sioux Falls, SD, the mice had Teklad Corn Cob Bedding (Harlan Laboratories, Indianapolis, IN) and paper enrichment (TEK-Fresh Cellulose, Harlan Laboratories, Indianapolis, IN). In both Sioux Falls, SD, and Rochester, NY, mice were weaned on postnatal day 21. All procedures were carried out according to the guidelines of the Animal Welfare Act and NIH policies. Animal studies carried out in Rochester, NY, and in Sioux Falls, SD, were approved by the University of Rochester Animal Care and Use Committee and the Sanford Research Animal Care and Use Committee, respectively.

Body weight measurement. Mice were weighed 20 minutes before the training session of the rotarod tests. Besides the weight of rotarod-tested mice, some of the data sets also contain weight measurements of mice not tested on the rotarod (Fig. 2a and Fig. 4). Different C57BL/6J mice were weighed at 1 and 5 months of age (Fig. 1 and Fig. 5).

Rotarod test. Accelerating rotarods (AccuScan Instruments, Inc., Columbus, OH; and Columbus Instruments, Columbus, OH) were used to measure the motor coordination of mice. The rotarod measures the ability of the mouse to maintain balance on a motor-driven, rotating rod. Thus, the fore- and hind limb motor coordination and balance can be analyzed.

Different C57BL/6J mice were tested on the rotarod at 1 and 5 months of age (Fig. 1 and Fig. 5).

One-month-old C57BL/6J mice, 0.4 rpm/s acceleration (Fig. 5a). The start speed of the rotarod was 0 rpm. The cut-off time was set at 200 s but all mice fell from the rotarod way before the set cut-off time. These mice were trained on the rotarod in four trials each consisting of two consecutive runs, with 15 min of rest between the trials. Following training, mice rested for 3 h and then were tested on the rotarod in four test trials each consisting of two consecutive runs, with 15 min of rest between the trials. The average rotating speed (rpm) when the mouse fell from the rotating rod in the 4 test trials was calculated for each mouse.

Five-month-old C57BL/6J mice, 0.3 rpm/s acceleration (Fig. 1b and Fig. 5b). The start speed of the rotarod was 0 rpm. The cut-off time was set at 120 s but all mice fell from the rotarod way before the set cut-off time. These mice were trained on the rotarod in three trials each consisting of two consecutive runs, with 15 min of rest between the trials. Following training, mice rested for 1 h and then were tested on the rotarod in four test trials each consisting of two consecutive runs, with 15 min of rest between the trials. After a 3-h rest interval, 5-month-old C57BL/6J mice in Sioux Falls were also tested in a 2nd rotarod test (Fig. 5b) using an increased acceleration (0.4 rpm/s, cut-off time set at 200 s) in four test trials each consisting of two consecutive runs, with 15 min of rest between the trials. The average rotating speed (rpm) when the mouse fell from the rotating rod in the test trials was calculated for each mouse.

One-month-old 129S6/SvEv mice, 0.1 rpm/s acceleration (Fig. 2b). The start speed of the rotarod was 0 rpm. The cut-off time was set at 240 s but most mice fell from the rotarod way before the set cut-off time. These mice were trained on the rotarod in three consecutive runs. Following training, mice rested for 1 h and then were tested on the rotarod in three test trials each consisting of three consecutive runs, with 15 min of rest between the trials. After a 3-h rest interval, the mice were tested on the rotarod again in three test trials each consisting of three consecutive runs, with 15 min of rest between the trials. The average rotating speed (rpm) when the mouse fell from the rotating rod in the test trials was calculated for each mouse.

One-month-old 129S6/SvEv mice, 0.2 rpm/s acceleration (Fig. 3c). The start speed of the rotarod was 0 rpm. The cut-off time was set at 240 s but all mice fell from the rotarod way before the set cut-off time. These mice were trained on the rotarod in three consecutive runs. Following training, mice rested for 1 h and then were tested on the rotarod in three test trials each consisting of three consecutive runs, with 15 min of rest between the trials. After a 3-h rest interval, the mice were tested on the rotarod again in three test trials each consisting of three consecutive runs, with 15 min of rest between the trials. The average rotating speed (rpm) when the mouse fell from the rotating rod in the test trials was calculated for each mouse.

One-month-old 129S6/SvEv mice, 0.4 rpm/s and 0.6 rpm/s accelerations (Fig. 4). The start speed of the rotarod was 0 rpm. These mice were trained on the rotarod at 0.2 rpm/s acceleration (cut-off time set at 100 s) in three trials each consisting of two consecutive runs, with 10 min of rest between the trials. Following training, mice rested for 3 h and then were tested on the rotarod at 0.2 rpm/s acceleration (cut-off time set at 100 s) in two test trials each consisting of two consecutive runs (with 10 min of rest between the two trials). After a 20-min rest interval, the mice were tested at 0.4 rpm/s acceleration (cut-off time set at 100 s) in two test trials each consisting of two consecutive runs (with 10 min of rest between the two trials). Following a 20-min rest interval, the mice were finally tested at 0.6 rpm/s acceleration (cut-off time set at 100 s) in two test trials each consisting of two consecutive runs, with 10 min of rest between the two trials. The average rotating speed (rpm) when the mouse fell from the rotating rod in the test trials was calculated for each mouse.

Statistical analysis. Most weight and rotarod data sets passed the normality test (alpha level 0.05), and therefore, unpaired, two-tailed t-test, 2-way ANOVA with Bonferroni's post-test, and repeated measures 2-way ANOVA with Bonferroni's post-test were applied in the statistical analysis using GraphPad Prism 5 and SigmaPlot 12 programs; alpha level was 0.05 in all cases. Unpaired, two-tailed t-test was used to compare the weight and motor coordination of 5-month-old male C57BL/6J mice in Rochester, NY and Sioux Falls, SD (Fig. 1); the weight of 1-month-old male and female 129S6/SvEv mice in Sioux Falls (Fig. 4); the weight and motor coordination of 1-month-old male and female C57BL/6J mice in Sioux Falls (Fig. 5a); and the weight of 5-month-old male and female C57BL/6J mice in Sioux Falls (Fig. 5b).

Two-way ANOVA with Bonferroni's post-test was applied to compare the weight of 1-month-old C57BL/6J males and females, respectively, in Rochester and Sioux Falls (Fig. 1a); the weight of 1-month-old 129S6/SvEv males and females, respectively, in Rochester and Sioux Falls (Fig. 2a); the weight and motor coordination of 1-month-old 129S6/SvEv males and females, respectively, on the two different diets in Sioux Falls (Figs. 3a and 3c); the weight of 1-month-old 129S6/SvEv males and females, respectively, on the 5010 diet in Rochester and Sioux Falls (Fig. 3b); the motor coordination of 1-month-old 129S6/SvEv males and females in Sioux Falls (Fig. 4); and the motor coordination of 5-month-old C57BL/6J males and females in Sioux Falls (Fig. 5b).

Repeated measures 2-way ANOVA with Bonferroni's post-test was used to compare the 1st and 2nd rotarod test results of the same 129S6/SvEv mice in Rochester and Sioux Falls, respectively (Fig. 2b); and the 1st and 2nd rotarod test results of the same 129S6/SvEv mice kept on the 5010 and 2018 diets, respectively in Sioux Falls (Fig. 3c).

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Author contributions

D.A.P. and A.D.K. designed the experiments. A.D.K. performed the weight measurement and behavioral testing in both Rochester, NY, and Sioux Falls, SD. D.A.P. and A.D.K. analyzed the data and wrote the manuscript.

Additional information

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