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Original Research

Choosing the Right Wild Type: Behavioral and Neurochemical Differences Between 2 Populations of Sprague–Dawley Rats from the Same Source but Maintained at Different Sites

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Sprague–Dawley rats are often the background stock for transgenic rats, and rats from various sources may differ in their physical development and behavior. In 1990, the Institute of Cytology and Genetics in Novosibirsk obtained Sprague–Dawley rats from a commercial vendor and bred them in a closed colony. To study various aspects of the physical development and behavior of male F1 progeny of the Novosibirsk colony (Nov:SD) and commercial colony (Crl:SD) raised in identical environments, we evaluated body weight; food and water consumption; behavior in the elevated-plus maze (X maze), open field, free exploration paradigm, hole board, and the rotarod; and serotonin content in brain regions. Crl:SD rats were heavier and consumed more food than did the Nov:SD rats, which displayed a higher level of motor activity in all tests without displaying differences in anxiety-related behavior in the X maze or open-field test. In the free exploration paradigm, more Nov:SD rats explored the outside and started exploration earlier; they also were more active and showed less habituation in the hole-board test. Brain serotonin content was higher in the Crl:SD rats. In conclusion, prolonged isolated breeding of 2 stocks of Sprague–Dawley rats led to populations that differed in their exploratory and anxiety-related behavior, physical development, and serotonergic neurotransmission. Therefore, rats of the same stock but obtained from different breeders should be used with caution in research involving these measures.

Abbreviation: 5HT, serotonin

Outbred rats are extensively used in pharmacologic and biologic research, despite considerable evidence of their increased phenotypic deviation and therefore preferential use of inbred strains.[8,16] Sprague–Dawley rats are the most widely used outbred rats in laboratory animal research (appearing in about 60,000 publications in Medline during the past 5 y compared with 50,000 publications featuring Wistar rats) and are being used increasingly in behavioral pharmacology. Further, Sprague–Dawley rats are often the background strain for transgenic rats including TGR(ASrAOGEN)680, a rat with specific downregulation of astroglial angiotensinogen synthesis,[56] TGR(mRen-2)27, a rat with an added mouse *renin 2* gene,[37] and a transgenic rat harboring the human vasopressin gene.[38] Many transgenic rats are studied in behavioral experiments,[40,66,68] in which their phenotype is compared with that of wild-type rats of the corresponding stock (outbred colonies of 1 rat strain are referred to as 'stocks,' whereas inbred colonies of 1 rat strain are called 'strains' or 'lines').[8]

If the possible effects of the genetic manipulation are under investigation, the wild-type littermates of transgenic rats ideally should be used as the controls. However, transgenic rats (and their wild-type littermates) are often kept in separate colonies for several generations, and whether the subsequent wild-type littermates differ from the original rats is unclear. Often rats of an outbred stock are purchased to use as wild-type controls, whereas the transgenic rats are bred in a closed colony elsewhere.[11,64] For example, "Experiments were performed in male heterozygous transgenic TGR(mREN2)27 rats…Normotensive Sprague–Dawley (SD) rats (Zentralinstitut für Versuchstierkunde, Hannover, Germany) were used as controls."[55]

To accurately determine the effects of the genetic manipulations on the animals' behavior, the behavior of the wild-type rats has to be assessed thoroughly. Interstrain differences in laboratory rats are particularly important to behavioral pharmacologists, whose studies depend on well-defined, homogenous backgrounds. Anxiety-related behavior is a major area of research in our laboratory;[5,49,50,52] in addition, this type of behavior is assessed routinely in the process of behavioral phenotyping of transgenic animals.[10] Selective alteration of genes is attempted to examine specific hypotheses about the behavioral function of a gene. The tests used for behavioral phenotyping are chosen in accordance with the hypothesis about the function of the gene of interest. A thorough knowledge of the behavioral literature and procedures is necessary to use an optimal test hierarchy. Interestingly, many transgenic animals show alterations of anxiety-related behavior compared with their respective wild type.[10]

Furthermore, anxiety-related behavior differs among substrains of inbred rats and stocks of outbred rats, subsequently potentially causing different and sometimes contradictory

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results with the same behavioral procedure or test.[23,29,49] Behavioral differences among stocks of rats have been described repeatedly.[9,28,49] However, the Sprague-Dawley rats used in the cited experiments were bred by various vendors, and phenotypic differences among rats from different sources could be expected. To gain insight into this problem, we wanted to examine aspects of physical development and behavior, focusing on anxiety-related behavior, in 2 Sprague–Dawley populations with common ancestry but bred at different sites. Our experiment addresses a common practice during the breeding of genetically modified rats, given that many smaller research facilities maintain the breeding of desired transgenic rats but obtain control animals from a commercial breeder.

More than 15 y ago, the animal facility of the Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences in Novosibirsk started breeding Sprague–Dawley rats obtained from a commercial source. No new animals have been added to this colony since it was established.[21,33] Because separate breeding of inbred strains for combined 20 generations (for example, since separation, the separated colony and its parent colony have each been bred for 10 generations—20 generations between the 2 at present) may give rise to a substrain,[2,22] breeding of the closed colony of Sprague–Dawley rats at Novosibirsk for more than 45 generations may have led to a population with various differences from those currently obtainable from the commercial source of the founders of the colony.

Because many transgenic rats are derived from Sprague–Dawley rats, we used the population from Novosibirsk as a model for segregated breeding of a small founder group, as often occurs with transgenic rats.[37] To assess possible differences in the general physical development between the population from Novosibirsk and animals currently available from the source that provided the founder animals for the Novosibirsk colony, we measured the body weight during the first 70 d and the amount of food and water consumed during 24 h.[10] For determination of anxiety-related behavior, motor function, and learning and memory, we used the elevated-plus maze (X maze),[36] open-field test,[46,67] a free exploration paradigm for assessment of 'trait anxiety',[25,49] the hole-board test (to evaluate exploration and habituation of the rats);[7,18] and the rotarod test, a classic method for evaluating motor coordination.[12,32] Based on the Y maze, the elevated-plus maze is used to assess 'state anxiety'-related behavior,[36] although underlying trait anxiety affects the rats' behavior in this test.[19,29] The open-field test is used widely to evaluate motor function and normal exploratory locomotion (horizontal and vertical movements).[46,67]

Serotonin (5HT) plays a crucial role in anxiety-related and feeding behaviors in animals and humans.[31,39] Several experimental studies have suggested that a reduction in the activity of 5HT in the brain is associated with anxiolysis, and other studies have shown that benzodiazepines inhibit the firing of serotonergic neurons in the midbrain raphe region, the brain structure that contains most of the serotonergic neurons which project to the limbic and cortical regions of the brain.[5,47,58,69] The strong relationship between serotonergic pathways and anxiety is supported by the effects of nonbenzodiazepine anxiolytics such as buspirone and tandospirone, which decrease central serotonergic function by stimulating $5HT_{1A}$ receptors.[23] In contrast, drugs that increase the activity of the central 5HT system have anxiogenic effects in animals and man.[23] We previously showed that the 5HT levels in discrete brain regions varied between strains differing in anxiety-related behavior: less-anxious rats had lower 5HT concentrations in the tissue than did anxious rats.[5,51] In view of this difference, in the current study we also assessed the 5HT tissue levels in CNS regions related to anxiety to assess possible differences in 5HT neurotransmission between the 2 rat populations.

Material and Methods

All procedures and experiments were performed according to the German Animal Protection law and were approved by the Berlin animal care and use committee.

Animals. Male outbred Sprague–Dawley rats were obtained from Charles River Laboratories Germany (Crl:SD; Sulzfeld, Germany) and the Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia (Nov:SD). Breeding of the Sprague–Dawley rats at Novosibirsk started in 1990 with 15 breeding pairs and was maintained at that size by using a rotational breeding system to keep the generations as heterogenous as possible.[13,53] Prior to our experiments, 45 generations had been raised, and no genetic material from other sources had been introduced.[33] After receiving the rats from both sources, we subsequently bred and raised F1 animals (Crl:SD, n = 20; Nov:SD, n = 26) under identical environmental conditions in our animal unit.

Environment. The animals were kept under conventional conditions at a room temperature of 22 ± 1 °C, 55% ± 10% relative humidity, and a 12:12-h light:dark cycle (lights on, 0600) with diffuse lighting of 105 to 125 lux.

Housing. After weaning, the rats were group-housed at 4 to 5 per cage (dimensions, 45 × 60 × 25 cm; Macrolon Type IV, Ehret, Emmendingen, Germany). For determination of food consumption and water intake, animals were single-housed (cage dimensions, 37.5 × 21.5 × 18 cm; Macrolon Type III, Ehret, Emmendingen, Germany) for 7 d starting at postnatal day 30. Tap water in drinking bottles and food pellets containing 19.0% protein, 13.5% water, 4.0% fat, 6% fiber, and 7% ash (Altromin 1326, Altromin, Lage, Germany) were freely available. Cages were changed twice weekly, and softwood bedding (Altromin 3/4, Altromin) was used.

Test environment. All behavioral tests were performed in soundproof chambers (180 × 180 × 230 cm) between 0800 and 1100. The rats were first tested when 50 d of age; group size was 10 to 12 animals. The animals were transferred in their home cages from the animal unit to the soundproof chambers 1 h before testing. During the experiments, the animals were observed from outside the room by use of a video camera suspended above the apparatus; their behavior was recorded and analyzed with a computerized automated tracking system (VideoTrack, CPL Systems, Cambridge, UK).

Experimental procedure. Rats were weighed on postnatal days 20, 30, 50, 64, and 78 by using an animal balance (LC 2200, Sartorius, Goettingen, Germany). On day 43, the voluntary food intake and water consumption during 24 h were determined.[10] On day 45, all animals were assigned randomly to 1 of 2 experimental groups (each group comprised 10 Crl:SD and 13 Nov:SD rats). On day 50, the rats were placed in either the X maze (group 1) or hole board (group 2); on day 64, the rats were placed either into the open field (group 1) or on the rotarod (group 2). Rats were assigned to 2 different groups to avoid a sustained habituation or learning effect induced by repeated testing in animal tests based on similar stimuli (open field and hole board). On day 78, all animals were tested in the free exploration paradigm.

X maze. The X maze was made from gray polycarbonate plastic illuminated with 210 lux on the surface of the open arms, 190 lux in the center, and 160 lux in the closed arms. The X maze was

64 cm high with 4 arms (44 × 15 cm; the wide arms are beneficial for the use of larger rats), with a wall (height, 15 cm) on each of 2 opposite arms.[27,29] On day 50 the animals were placed in the center of the X maze facing a corner between an open and a closed arm. The experiments were performed for 5 min.[45,54] The classic anxiety-related behavioral parameters measured were the numbers of entries into closed arms and open arms (with all 4 paws in an arm) and the time spent in the open arms.[44,45] The numbers of head dips over the edge of the open arms and of stretched attended postures (animal stretches forward from a protective, closed arm into a more-aversive open arm without leaving the closed arm and then retracts to its original position) are considered to be parameters of risk assessment.[54] The percentage of entries into open arms was calculated. The total distance traveled (in meters) and the number of rearings were determined as measures of locomotor activity.[45]

Open field. On day 64 the rats were placed in the center of a brightly illuminated (350 lux) white open field (100 × 100 × 40 cm). Each rat was observed for 5 min; the time spent within 16 cm of the walls of the open field and the time the animals spent grooming themselves (a calming behavior[61]) were determined. The total distance traveled in meters and the number of rearings were determined as measures of locomotor activity.

Free exploration paradigm. The free-exploration paradigm was performed in the familiar surroundings of the moderately illuminated (100 lux) animal unit.[25,49] The home cage was placed on a stand in the animal room, and the lid was removed and placed to facilitate the rats' exiting from the cage. The latency to leave the cage and the percentage of animals that explored outside the home cage during the first 10 min were determined.

Hole-board test. On 2 consecutive days (days 50 and 51), the rats were placed for 10 min in a black polycarbonate plastic box (50 × 50 cm; walls 30 cm high) with 16 equally spaced holes (diameter, 2.5 cm; 10 cm apart) in the floor. The number of head dips was recorded. A reduction in the number of head dips on the second day was interpreted as habituation to an unfamiliar environment.[20] The ratio of the number of head dips on the second day to that on the first day was expressed as a percentage. The total distance traveled (in meters) and the number of rearings were determined as measures of locomotor activity.[20]

Rotarod test. In a separate experiment, motor coordination was evaluated with an accelerating rotarod for rats (TSE, Bad Homburg, Germany).[12,32] The treadmill consisted of 4 rotating drums (diameter, 7 cm; 24 cm above ground), divided by flanges. On day 64, rats were familiarized with the apparatus; they were placed for three 2-min runs on the constantly revolving drum (speed, 4 rpm). If a rat fell off during the trials, it was immediately placed back onto the drum. On the next day (day 65), the rats were placed on the accelerating rotating drums (speed, 4 to 32 rpm) for a maximum of 5 min. The latency until falling off the drum was recorded for each rat.

Determination of 5HT tissue levels. The animals were sedated with isoflurane (Forene, Abbott, Germany) and decapitated, and the brains were removed and immediately frozen in liquid nitrogen. Subsequently, sagittal sections (thickness, 1 mm) of the prefrontal cortex (bregma 3.2 mm), hippocampus (bregma 5.8 mm), and the dorsomedial raphe nucleus (bregma 7.8 mm)[43] were made, and the regions of interest dissected by using a 1-mm tissue punch (Hauptner-Herberholz, Solingen, Germany). The samples were weighted, immersed in ice-cold 0.1 M perchloric acid (600 µl), homogenized, and centrifuged for 10 min (16,250 × *g* at 5 °C). The concentration of 5HT in the supernatant was determined by high-performance liquid chromatography with electrochemical detection.[5] Concentrations of 5HT are given as nanograms per milligram of wet tissue.

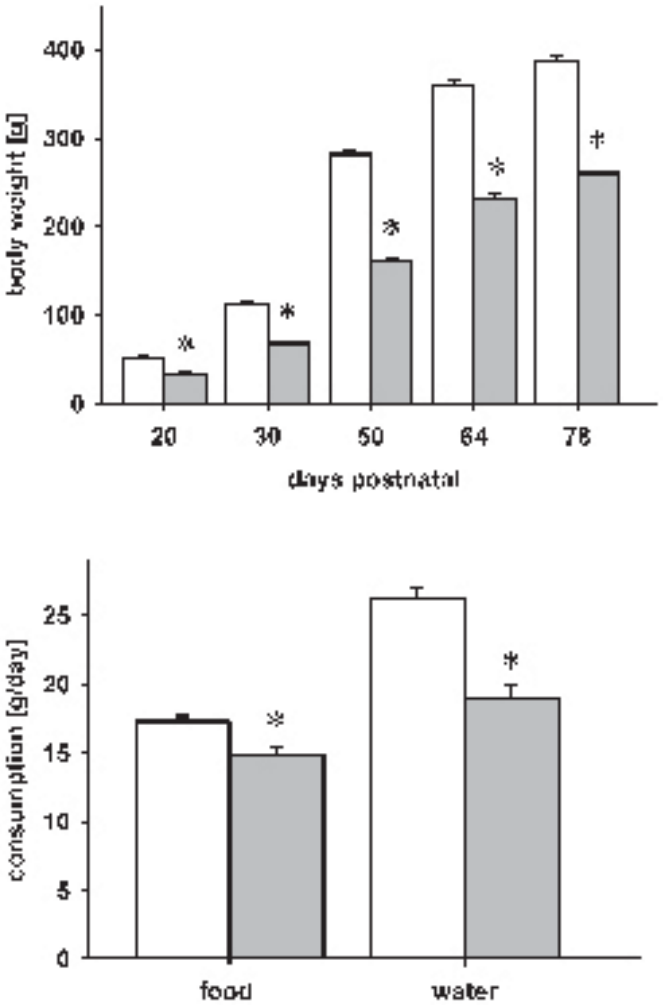

Figure 1. Body weight over time and 24-h food and water consumption (mean ± standard error of the mean; n = 20 to 26) of 45-d-old Sprague–Dawley rats from a commercial vendor (Crl:SD, open bars) and Novosibirsk (Nov:SD, shaded bars). *, $P < 0.05$ (Student *t* test) between Crl:SD and Nov:SD.

Statistics. Statistical analysis of the behavioral data was performed by using Student *t* or Mann–Whitney rank-sum tests as appropriate. Body weight data underwent 2-way analysis of variance followed by the Holm–Sidak test. Data are presented as mean ± standard error of the mean. The exploration data from the free exploration paradigm were analyzed by using the Fisher exact test; the results are given as a percentage. A *P* value of less than 0.05 was considered statistically significant. Statistical analysis was performed using SigmaStat for Windows V3.01 (Systat Software GmbH, Erkrath, Germany).

Results

Body weight, food intake, and water consumption. The body weight of the Crl:SD rats differed markedly from that of the Nov:SD rats (F[df 1] = 735.754, $P < 0.001$]. Starting at postnatal day 20, the body weight of the Crl:SD rats was persistently greater (t[df 1] = 3.122, $P < 0.05$] than that of the Nov:SD rats until the day 78 (t[df 1] = 17.398, $P < 0.05$; Figure 1). Food intake during 24 h was greater for Crl:SD rats than Nov:SD rats (t[df 22] = 3.328, $P < 0.05$); Crl:SD rats also drank more water (t[df 22] = 3.627, $P < 0.05$; Figure 1).

X maze. Anxiety-related behavior after exposure to the X

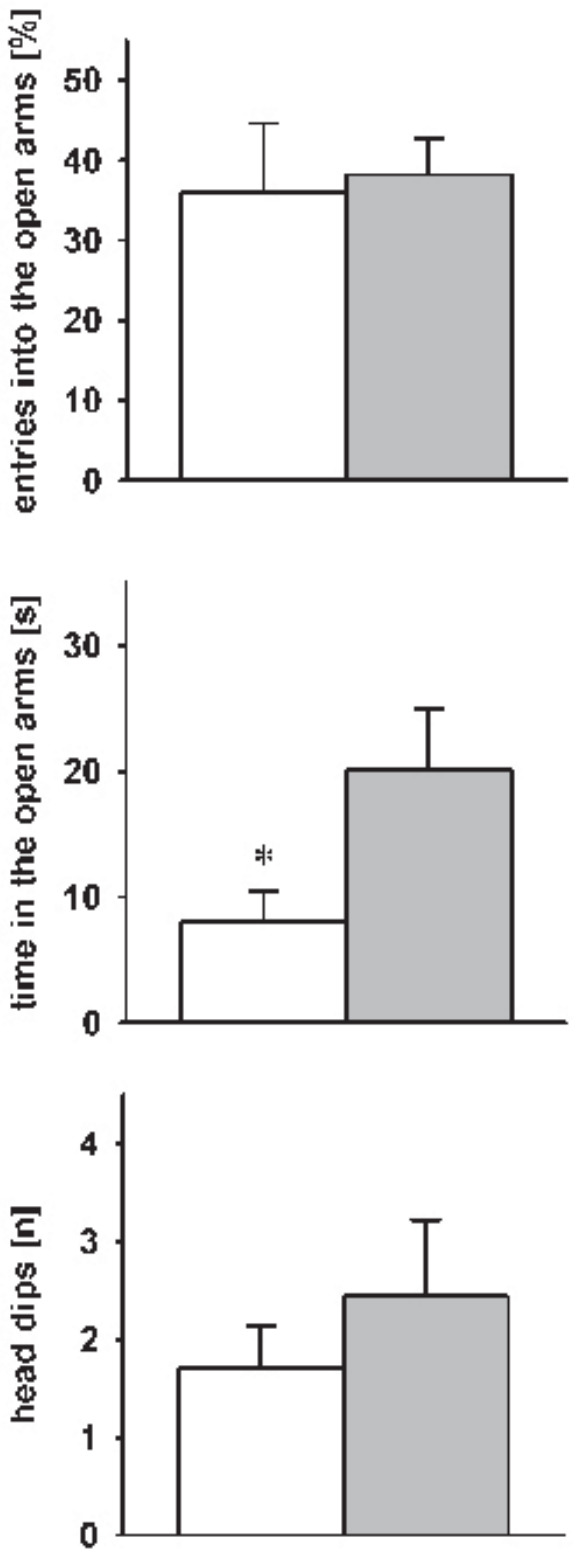

Figure 2. Behavior of Sprague–Dawley rats from a commercial vendor (Crl:SD, open bars) and Novosibirsk (Nov:SD, shaded bars) on the elevated-plus maze. *, $P < 0.05$ (Student *t* test) between Crl:SD and Nov:SD. Data are presented as mean ± standard error of the mean (n = 10 to 13).

maze did not differ markedly between the 2 rat populations. Although the percentage of entries directed into the open arms did not differ, the Nov:SD rats spent more time in the open arms than did the Crl:SD rats (t[25] = 6.748, $P < 0.05$; Figure 2). The number of stretched attended postures (mean ± standard error of the mean) did not differ between Crl:SD rats (0.6 ± 0.2) and the Nov:SD rats (0.8 ± 0.3), nor did the number of returns to the protected closed arms (1.2 ± 0.3 and 2.1 ± 0.4, respectively). During exploration in the open arms, the Crl:SD rats showed fewer head dips than did the Nov:SD rats (1.7 ± 0.5 versus 2.4 ± 1.1, t(25) = 0.5793, $P < 0.05$; Figure 2). Both populations differed markedly in the number of closed entries in the X maze (Crl:SD rats, 4.3 ± 1.1; Nov:SD rats, 12.8 ± 1.0; t(25) = 5.707, $P < 0.05$) and in the distance traveled during the test (t[25] = 5.616, $P < 0.05$; Table 1). The number of rearings, an indicator of vertical exploration, were similar between the Crl:SD and Nov:SD rats (Table 1).

Table 1. Locomotor and vertical exploratory activity (rearing) of Sprague–Dawley rats from Novosibirsk (Nov:SD) and a commercial vendor (Crl:SD) in X-maze, open-field, and hole-board tests

Test	Population	Locomotor activity (min)	No. of rearings
X maze	Crl:SD	8.1 ± 1.0	9.6 ± 1.2
	Nov:SD	15.2 ± 0.8	18.7 ± 1.0[a]
Open field	Crl:SD	9.2 ± 1.5	8.5 ± 1.4
	Nov:SD	21.4 ± 1.4	20.6 ± 1.5[a]
Hole board	Crl:SD	10.8 ± 1.3	14.9 ± 2.2
	Nov:SD	17.6 ± 1.2	30.7 ± 3.4[a]

Data are expressed as mean ± standard error of the mean (n = 10 to 13 per group).
[a]$P < 0.05$ (Student *t* test) compared with value for Crl:SD.

Open field. In the open-field test, the tendency to remain close to the walls, an index of anxiety-related behavior, did not differ significantly between Crl:SD rats (295.7 ± 3.6 s) and Nov:SD rats (284.3 ± 6.1 s; Figure 3). In addition, the duration of self-grooming was similar between Crl:SD rats (40.1 ± 7.7 s) and Nov:SD rats (36.7 ± 5.5 s; Figure 3). However, the rats from the 2 populations differed in the distance traveled and number of rearings, an indicator of vertical exploration. Compared with the Nov:SD rats, Crl:SD rats traveled less (t[38] = 5.946, $P < 0.05$) and showed fewer rearings (t[38] = 4.956, $P < 0.05$; Table 1).

Free exploration paradigm. In the free exploration paradigm, few Crl:SD rats explored outside of their familiar home cage. Whereas a few of the Crl:SD rats explored on the first day of testing (day 78), most of the Nov:SD rats left the home cage ($P < 0.05$). The latency to leave the cage was longer for Crl:SD rats than Nov:SD rats (t[38] = 4.391, $P < 0.05$; Figure 3). During the next 2 d (days 79 and 80), the percentage of exploring Crl:SD rats did not vary significantly, whereas the percentage of exploring Nov:SD rats increased ($P < 0.05$). Consequently, the latency to leave the cage decreased markedly in the Nov:SD rats compared with the Crl:SD rats (t[38] = 3.914, $P < 0.05$; Figure 3).

Hole board. In the hole-board test, the behavior of rats from the 2 populations differed markedly. During the first exposure to the hole board, Crl:SD rats explored less than did Nov:SD rats (t(18) = 4.285, $P < 0.05$; Figure 4), and on the second day, the Nov:SD rats showed more head dips than did the Crl:SD rats (t[18] = 4.285, $P < 0.05$). However, the Nov:SD rats did not habituate to the unfamiliar environment of the hole-board apparatus, shown by the lack of reduction of head dips on the second day (74.9% ± 5.9%), whereas the amount of head-dipping among Crl:SD rats on the second day of testing was decreased significantly (40.3% ± 4.6%, t[18] = 3.502, $P < 0.05$). In addition, the 2 populations differed in the distance traveled and number of rearings. During the first exposure to the hole board, Crl:SD rats traveled less (t[18] = 3.844, $P < 0.05$; Table 1) and showed less rearing activity (t[18] = 4.639, $P < 0.05$; Table 1) than the Nov:SD rats. Similar differences were present on the second day, when

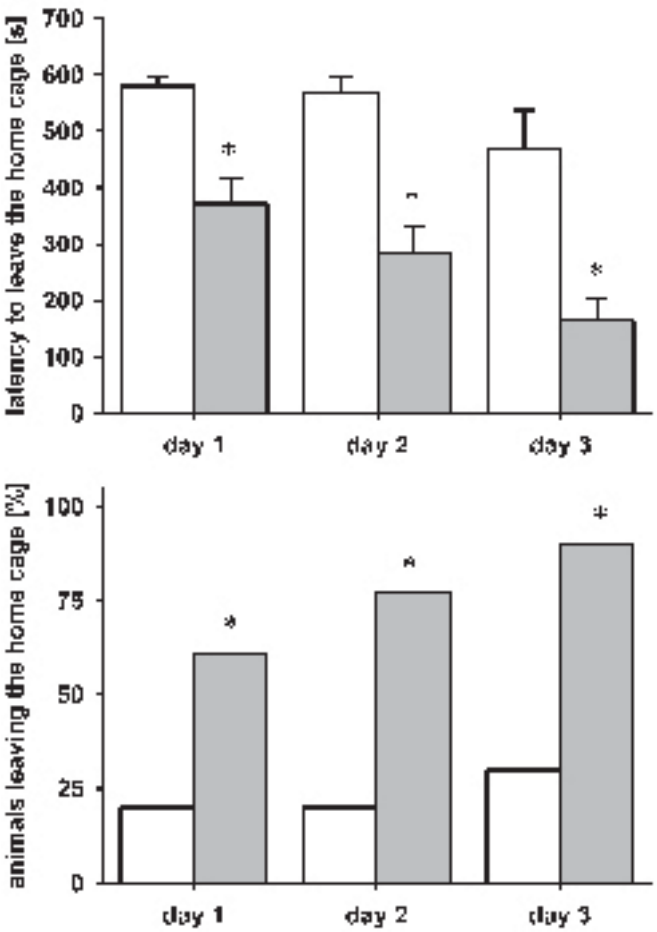

Figure 3. Free exploration behavior of Sprague–Dawley rats from a commercial vendor (Crl:SD, open bars) and Novosibirsk (Nov:SD, shaded bars) under familiar conditions. Data showing latency to leave the home cage voluntarily were analyzed using the Student *t* test (*, $P < 0.05$ between Crl:SD and Nov:SD). Data regarding rats exploring surroundings outside the home cage were analyzed using Fisher's exact test (*, $P < 0.05$ between Crl:SD and Nov:SD). Data are presented as mean ± standard error of the mean (n = 10).

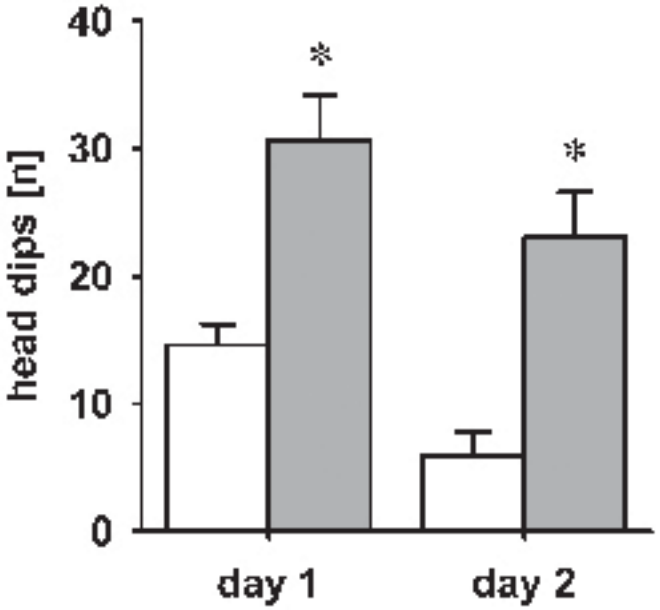

Figure 4. Habituation of exploratory behavior of Sprague–Dawley rats from a commercial vendor (Crl:SD, open bars) and Novosibirsk (Nov:SD, shaded bars) in the hole board. Data are shown as the total number of head dips on 2 consecutive days. *, $P < 0.05$ (Student *t* test) between Crl:SD and Nov:SD. Data are presented as mean ± standard error of the mean (n = 10 to 13).

Crl:SD rats again traveled less (t(18) = 3.585, $P < 0.05$; Table 1) and showed less rearing activity (t[18] = 5.755, $P < 0.05$; Table 1) than the Nov:SD rats.

Rotarod test. In the rotarod test, latency to falling off the drum was shorter for the Crl:SD rats (106.2 ± 1.5 s) than the Nov:SD rats (154.7 ± 7.9 s, t[26] = 3.158, $P < 0.05$), suggesting diminished motor coordination in the Crl:SD rats.

Determination of 5HT tissue levels. The Crl:SD rats had lower 5HT tissue levels in the prefrontal cortex ($P < 0.05$, T = 75), hippocampus ($P < 0.05$, T = 59), and in the region of the raphe nuclei ($P < 0.05$, T = 71) than did the Nov:SD rats (Figure 5).

Discussion

Sprague–Dawley rats are raised all over the world. Although all Sprague–Dawley rats stem from those that originated at the Sprague–Dawley Company (Madison, WI) in 1925, several reports in the literature indicate that Sprague–Dawley rats obtained from different commercial vendors show various differences in, for example, noradrenergic innervations of the spinal cord,[41] respiratory control mechanism,[59] and neuropathic pain behavior.[72] Unlike the animals in the cited studies, the rats from the 2 colonies we examined originated from the same vendor. However, our results show that even rats originating from the same population, but bred separately for 15 y, differ in their physical development, motor behavior, and anxiety-related behavior. Separate breeding of inbred strains is well known to give rise to substrains.[22] Rats from outbred stocks have a wider genetic heterogeneity than do inbred strains and supposedly represent an analog of outbred populations, such as humans. The genetic variability in outbred rats and mice may range from near genetic homogeneity to a colony with high heterogeneity.[16,17] However, the extent of the genetic variation within a group of outbred rats is not known in general and can be determined only by using molecular biology techniques. The scientific value of experiments using outbred rats is under discussion.[8,16,17]

Some of the behavioral differences possibly detected in rats of the same stock or strain but obtained from different vendors might be due to differences in breeding conditions, handling, or housing.[70,71] To avoid various vendor-specific effects,[49] we used F1 Sprague–Dawley rats that we bred and raised in our animal unit. Although they were bred and raised under identical conditions, the 2 populations still differed noticeably in their exploratory behavior, some measures of anxiety-related behavior, food intake, and body weight throughout the observation period. The reduced food intake in the Nov:SD rats coincidences with their decreased body weight but caused no retardation or general behavioral impairment, as seen in the exploration-based experiments and rotarod test. Perhaps differences in the formulation of the rat diet between Novosibirsk and Germany and food shortages may act as a strong selective factor at these sites, but discussions with senior scientists at both animal centers failed to reveal any evidence for malnutrition at either institution.[21,33]

Humans are suggested to have 2 types of anxiety—trait anxiety and state anxiety—where state anxiety is a temporary emotional state, and may vary from task to task, and trait anxiety is the reflection of stable individual differences in the tendency to respond with state anxiety in the anticipation of threatening

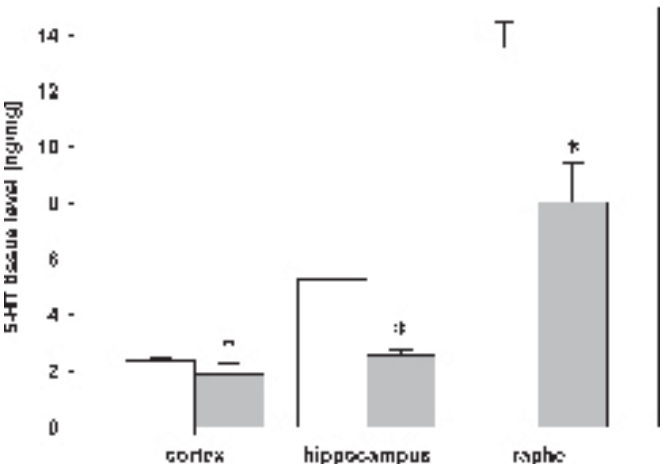

Figure 5. Concentrations of serotonin in the prefrontal cortex, ventral hippocampus, and raphe region of Crl:SD rats (open bars) and Nov:SD rats (shaded bars), as measured by high-performance liquid chromotography. *, $P < 0.05$ (Mann–Whitney *U* test) between Crl:SD and Nov:SD. Data are presented as mean ± standard error of the mean (n = 10).

situations.[60]

We studied both populations of rats in the X maze and open field to estimate their state anxiety in novel and aversive conditions and in the free exploration paradigm to assess trait anxiety among our animals. The X maze is well established[27,45,47] as a validated and reliable method for detecting both anxiolytic and anxiogenic effects of drugs[44] and is the most widely used animal test for anxiety.[29]

In the X maze, the 2 populations of rats showed no differences in the percentage of open-arm entries, a parameter reflecting anxiety-related behavior.[45] One reason for the extended stay of the Novosibirsk rats in the more aversive open arms could be their increased motor activity. Similar results were achieved previously after treatment with amphetamine.[48] In contrast; the X maze is mainly a model for state anxiety, although underlying trait anxiety will affect the behavior of rats during their exposure to the aversive conditions of the maze. Experiments with selectively bred rats suggest that the time spent in the open arms may be an indicator for trait anxiety.[57] Under these assumptions, the Nov:Crl rats appear to have less trait anxiety; the results from the free exploration paradigm confirm this assumption.

The open-field test is used widely to evaluate exploratory locomotion, motor function, and anxiety-related behavior.[3,10] Investigators need to remember that the open field is not a direct test of anxiety-related behavior,[26,46,67] but rather that patterns of exploration in the open field are influenced by anxiety.[1] In our open-field test, anxiety-related behavior was similar between the 2 rat populations despite the higher level of motor activity in the Nov:SD rats. This finding is in line with our results from the X maze, where the number of closed-arm entries was regarded as a measure of motor activity.

The results from the X maze and open-field test suggest that rats from the 2 populations do not differ in state anxiety. In contrast, in the free exploration paradigm, a prototypical test for trait anxiety in mice[4,24] and rats,[49] the Nov:SD rats explored the unknown surroundings of the familiar home cages earlier and more often, whereas the neophobic responses exhibited by the Crl:SD rats indicated a lower level of trait anxiety[3] in the Novosibirsk colony. A similar dissociation between state-anxiety-related behavior and the behavior in the free exploration paradigm occurred in outbred Wistar rats obtained from different vendors.[49]

5HT plays an important role in the control of anxiety. Increased activity and extracellular levels of 5HT are associated with anxiety-like behavior in the elevated-plus maze and the Geller–Seiffter test, whereas reduced 5HT synthesis, turnover, and release can induce anxiolytic-like behaviour.[5,69] In our study, the 5HT levels in selected brain areas were higher in the Crl:SD rats than the Nov:SD rats. Our present results correspond to earlier studies in which similar differences in 5HT levels in the central nervous system occurred in rats with high and low anxiety according to the social interaction test[51] and the elevated-plus maze.[5,58]

In addition to their differences in trait anxiety, the 2 populations differed markedly in their exploration and habituation in the hole-board test.[7] Unlike the Crl:SD rats, the Nov:SD rats did not habituate to the hole board. Habituation to the hole board, determined as decreased hole poking during the second exposure, measures the ability to remember having been in the same environment previously.[20] Therefore the Crl:SD rats seemed to learn or recall what they learned better than did the Nov:SD rats. However, in preliminary experiments using the inhibitory avoidance learning task, an established and thoroughly validated test of learning and memory in rats[65] and mice,[30] Crl:SD rats did not perform better than did Nov:SD rats, indicating the lack of learning impairment in the Nov:SD rats. Because changes in locomotion affect the frequency of nose-poking in the hole board, the difference in behavior between the Crl:SD and Nov:SD rats in the hole-board test could be caused by their divergent basal levels of motor activity.

Our investigation reveals that 2 populations of rats of the same stock and with a common origin but bred separately for 16 y differ in aspects of their anxiety-related behavior, motor activity, and body weight. How might the differences in trait anxiety and habituation to a new environment have arisen? Because the F1 progeny we tested were bred and raised under the same environmental conditions, their phenotypic differences suggest genetic variation between the 2 rat populations. Commercial vendors use random breeding schemes that avoid crosses between closely related animals to maintain the outbred population as heterogenic as possible. It is usually recommended that new colonies are set up with at least 25 breeding pairs. In smaller populations, the inevitable creep toward genetic homogeneity is delayed by the use of a rotational breeding scheme[13,15,53] with not less than 13 breeding pairs.[8] However, isolated breeding of a small group of 15 breeding pairs for more than 15 y (about 45 generations) could lead to the development of a discrete, 'not-quite-inbred,' population.[8,62]

Alternatively, the founder effect[34,35] might be responsible for the observed differences between the 2 populations of rats. Because the number of founder animals in the Novosibirsk colony was relatively small, it cannot be excluded that this population carried only a small fraction of the genetic variation present in the commercial vendor's colony and therefore was, just by chance, different from the larger population present in Germany. As a result, the Novosibirsk colony could be genetically and phenotypically different from the commercial population from which it was derived: over the last 16 y, the 2 populations could have diverged from each other. In the end, variations originally present within the 2 populations might now emerge as divergence between them.[35,62] The rate of genetic drift in a population depends strongly on population size, with small populations drifting more quickly than larger populations.[15] The relatively small size of the Novosibirsk colony could lead to accelerated genetic drift, compared with that of the larger commercial colony. Genetic drift seems to have a greater effect

in outbred stocks than in inbred strains.[14,28,42,63]

In our opinion the Novosibirsk population of Sprague–Dawley rats was a good model for transgenic animals with a genetic Sprague–Dawley background. For example, the TGR(mRen-2)27 rat strain that carries a mouse *renin 2* gene was founded with 3 females[37] and has been bred for the last 17 y (>50 generations) at the Max Delbrück Center for Molecular Medicine in Berlin. To our knowledge, this procedure for breeding transgenic rats is fairly common practice.

Overall, the Sprague–Dawley rats from the Novosibirsk colony seem to be different from commercially available Sprague–Dawley rats. These differences likely reflect contributions from all of the factors we have discussed. For the future we are planning a genetic analysis to assess the heterogeneity of the 2 populations we addressed here and subsequent assessment of the probable genetic divergence between them.

In conclusion, the data we have presented suggest various stock- and task-specific differences in the behavior of outbred rats. The appropriate choice of animals should reflect sufficient knowledge of behavioral genetics and the specific goals of the study. The use of inbred rats could overcome these serious limitations in the experimental use of animals,[6] but in research using transgenic rats, inbred transgenic animals would be necessary.

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