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Sensorimotor phenotyping of adult male and female *Tph2*-knockout rats

S.P. Konovalova [1], D.D. Shitc [2], Y.I. Sysoev [3], A. Vetlugina [1], M.M. Puchik [4], S.V. Okovityi [5], R.R. Gainetdinov [2], P.E. Musienko [1, 2, 3]

[1] Department of Neurobiology, Sirius University of Science and Technology, Sirius Federal Territory, Krasnodar region, Russia
[2] Institute of Translational Biomedicine, St. Petersburg State University, St. Petersburg, Russia
[3] Laboratory of Motor and Visceral Function Neuromodulation, Pavlov Institute of Physiology of the Russian Academy of Sciences, St. Petersburg, Russia
[4] Decision and Awareness Group, Cognitive Neuroscience Laboratory, German Primate Center – Leibniz Institute for Primate Research, Göttingen, Germany
[5] Department of Pharmacology and Clinical Pharmacology, St. Petersburg State Chemical and Pharmaceutical University, St. Petersburg, Russia
musienko.pe@talantiuspeh.ru

Abstract. The serotonergic system plays a critical role in sensorimotor control through the modulation of spinal circuits governing locomotor rhythm, posture, sensory processing, and muscle tone. Since tryptophan hydroxylase type 2 knockout (TPH2-KO) rats were developed to study the physiological role of central serotonin, research has been primarily focused on emotional and social behaviors, leaving sensorimotor functions largely unexplored. This study is aimed to provide a comprehensive phenotyping of sensorimotor behavior in TPH2-KO rats compared to their wild-type (WT) counterparts. Experiments were conducted on male (10–12 months old; TPH2-KO, $n = 12$; WT, $n = 9$) and female (12–16 months old; TPH2-KO, $n = 11$; WT, $n = 15$) Dark Agouti rats. The locomotor function was assessed via kinematic analysis to evaluate spatiotemporal gait parameters, postural control, and both interlimb and intralimb coordination. Motor coordination was tested using the Horizontal Ladder test, while sensory sensitivity was assessed with the Hot/Cold Plate test. Forelimb muscle strength was evaluated using the Grip Strength test. Our findings reveal that brain serotonin deficiency due to the *Tph2* gene knockout does not impair motor coordination, thermal sensitivity, or forelimb grip strength. However, a key phenotypic difference was observed in locomotion: TPH2-KO rats exhibited significantly higher locomotion speeds compared to WT animals. This increased speed accounted for secondary alterations in several kinematic gait parameters. These results suggest that while congenital central serotonin deficiency does not broadly affect basic sensorimotor functions, it specifically predisposes rats to adopt a faster locomotor gait, which in turn modifies their kinematic profile.

Key words: locomotion; gait analysis; sensorimotor function; rats; serotonergic system; *Tph2* knockout

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Сенсомоторное фенотипирование взрослых самцов и самок крыс с нокаутом гена триптофан-гидроксилазы 2-го типа

С.П. Коновалова [1], Д.Д. Шиц [2], Ю.И. Сысоев [3], А. Ветлугина [1], М.М. Пучик [4], С.В. Оковитый [5], Р.Р. Гайнетдинов [2], П.Е. Мусиенко [1, 2, 3]

[1] Направление нейробиологии, Научно-технологический университет «Сириус», Федеральная территория «Сириус», Краснодарский край, Россия
[2] Институт трансляционной биомедицины, Санкт-Петербургский государственный университет, Санкт-Петербург, Россия
[3] Лаборатория нейромодуляции двигательных и висцеральных функций, Институт физиологии им. И.П. Павлова Российской академии наук, Санкт-Петербург, Россия
[4] Группа исследований принятия решений и осознания, Лаборатория когнитивной нейронауки, Немецкий центр приматологии – Институт исследований приматов им. Лейбница, Гёттинген, Германия
[5] Кафедра фармакологии и клинической фармакологии, Санкт-Петербургский государственный химико-фармацевтический университет, Санкт-Петербург, Россия
musienko.pe@talantiuspeh.ru

Аннотация. Серотонинергическая система критически важна для выполнения сенсомоторных функций благодаря модуляции спинномозговых сетей, участвующих в контроле ритмики движений, позы, сенсорных функций, а также регуляции тонуса мышц. Для изучения физиологической роли серотонина в ЦНС предложены крысы с

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нокаутом гена триптофан-гидроксилазы второго типа (TPH2-KO). На сегодняшний день опубликовано много работ, описывающих изменения эмоционально-тревожного и социального поведения у таких животных, однако детального фенотипирования сенсомоторных функций еще не было проведено. В настоящей работе мы сравнили сенсомоторное поведение крыс TPH2-KO и дикого типа (WT). Использовали самцов (TPH2-KO, *n* = 12; WT, *n* = 9) 10–12 месяцев и самок (TPH2-KO, *n* = 11; WT, *n* = 15) 12–16 месяцев линии Dark Agouti. Оценивали локомоцию (кинематический анализ), моторно-координационную функцию (тест «Горизонтальная лесенка»), сенсорную чувствительность («Горячая/холодная пластина») и мышечную силу передних конечностей («Сила хвата»). Установлено, что нокаут гена триптофан-гидроксилазы 2-го типа не оказывает влияния на моторно-координационную функцию и температурную чувствительность конечностей, а также на мышечную силу передних лап у крыс. Важной особенностью локомоторного поведения у крыс TPH2-KO была бóльшая скорость по сравнению с животными дикого типа. Эта особенность определяла отличия нокаутных животных по ряду кинематических характеристик локомоции. Полученные данные свидетельствуют о том, что врожденное нарушение центральной серотонинергической передачи у крыс не сказывается на большинстве сенсомоторных функций, однако такие животные склонны к передвижению с большей скоростью, что может быть причиной изменения ряда кинематических характеристик локомоции.

Ключевые слова: локомоция; анализ походки; сенсомоторные функции; крысы; серотонинергическая система; нокаут *Tph2*

Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is a critical monoamine neurotransmitter in the central nervous system (CNS), which plays a role in the regulation of several physiological functions, including motor control (Kawashima, 2018). It is a key modulator of the central pattern generator, a group of interconnected spinal neurons involved in organizing stereotypical automatic movements (Gackière, Vinay, 2014). For instance, in experiments on isolated neonatal rat spinal cords, serotonin induced rhythmic activity in motoneurons and regulated their discharge parameters through actions on interneurons (Cazalets et al., 1992). In another study, which used a decerebrate cat model, serotonin was shown to modulate locomotion via 5-HT_7, 5-HT_{A2}, and 5-HT_{1A} receptors on neurons in spinal cord laminae VII and VIII, coordinating rhythmic movements and regulating the excitability of locomotor networks (Noga et al., 2009). In experiments on decerebrate rats, blockade of 5-HT_7 receptors disrupted coordination and reduced the amplitude of rhythmic activity induced by stimulation of the mesencephalic locomotor region (Cabaj et al., 2017). A specific combination of pharmacological agents targeting serotonin (5-HT_{1A}, 5-HT_7, 5-$HT_{2A/C}$), dopamine (D_1), and noradrenaline receptors (α2) not only restored rhythmic stepping activity with full weight support but also significantly improved balance control and fine motor skills, collectively bringing the locomotion of spinal rats as close to normal as possible (Musienko et al., 2011). Experiments on cats have shown that the serotonergic system (via 5-$HT_{2/7}$ receptors) is necessary for full-fledged locomotion induced by epidural spinal cord stimulation (Gerasimenko et al., 2009).

Serotonin is synthesized in the brain primarily by neurons of the raphe nuclei, where the key rate-limiting enzyme is tryptophan hydroxylase 2 (TPH2), an isoform specific to serotonergic neurons (Walther et al., 2003). The *Tph2* gene serves as the main target for studying the functions of serotonin in the CNS. In animal experiments with *Tph2* gene knockout, significant changes in emotional and cognitive domains have been documented. The animals exhibit increased aggressiveness and reduced anxiety levels (Mosienko et al., 2012; Meng et al., 2022), as well as impaired social behavior and diminished decision-making abilities, particularly under conditions of uncertainty and complex choice. Additionally, researchers have noted increased impulsivity (Alonso et al., 2024). However, the impact of congenital central serotonin deficiency on sensorimotor functions remains underexplored and is a subject of debate. Studying motor functions on an animal model with *Tph2* gene knockout is promising. It allows for an assessment of the contribution of central endogenous serotonin to the organization and control of motor acts, which may facilitate the development of new neurorehabilitation strategies for spinal cord injuries.

In a previous study (Sysoev et al., 2025), the effect of *Tph2* gene knockout on recovery following spinal cord hemisection was assessed using a limited set of behavioral tests, a small sample size (6 WT and 6 KO), and only female rats, which did not allow for a full identification of baseline differences between genotypes. Given the crucial role of serotonin in regulating sensorimotor functions and embryonic development, we conducted a detailed phenotyping of adult rats with *Tph2* knockout using a comprehensive battery of tests to assess locomotor and sensorimotor functions. Furthermore, this study was performed on animals of both sexes to identify gender-specific characteristics.

Materials and methods

Animals. All procedures were conducted in accordance with recommendations of the Council of the European Community (Directive 2010/63/EU of September 22, 2010) and the Rules of Good Laboratory Practice of the Eurasian Economic Union in the field of medicinal products (No. 81 of November 3, 2016). The animal study protocols were approved by the Ethics Committee of the St. Petersburg State University (St. Petersburg, Russia) (opinion No. 131-03-4 of April 3, 2023).

The animals were housed under standard vivarium conditions with controlled temperature (+22 °C) and a 12:12 light schedule (light from 8:00 to 20:00). Food and water were provided ad libitum. The study was performed in Tph2-deficient rats on Dark Agouti background (Kaplan et al., 2016) aged 12–16 months weighing 200–250 g. The experimental groups of male rats included 12 animals with *Tph2* gene knockout (TPH2-KO) and 9 wild-type (WT) rats, and the groups of female rats included 11 TPH2-KO and 15 WT (Fig. 1). Heterozygous animals (TPH2-HET) were not included in the analysis, because a previous study (Kaplan et al., 2016) had

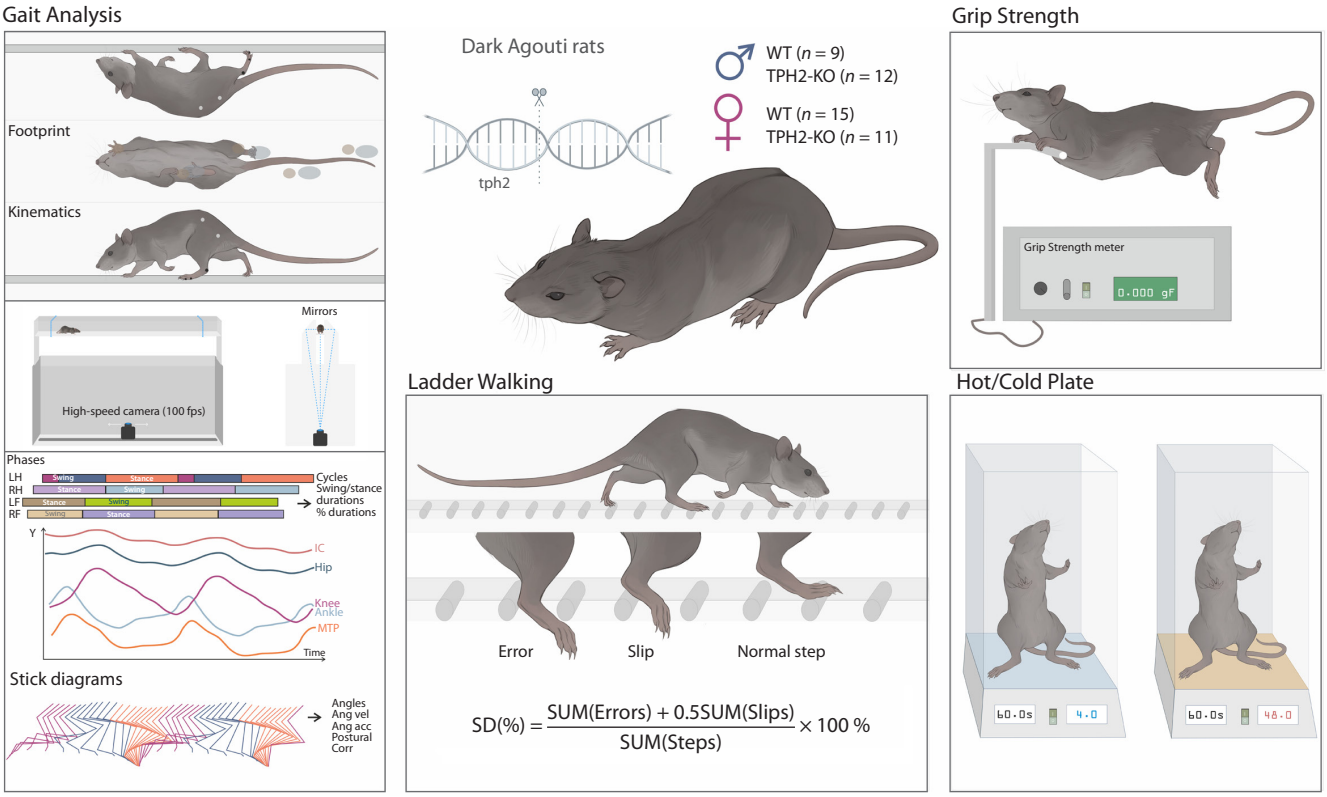


Fig. 1. Experimental animals and experimental design.

Left to right, top to bottom: an image of a rat traversing the MotoRater setup; group composition for experimental tryptophan hydroxylase 2 knockout (TPH2-KO) animals; a schematic of the Grip Strength test; a schematic of the MotoRater setup and the steps of data processing in the system's software, including gait phase analysis and the construction of linear topological diagrams (stick diagrams) of traversals; a schematic of the Horizontal Ladder test with an illustration of possible paw positions of the rat and the formula for calculating sensorimotor deficit (SD); schematics of the Cold Plate and Hot Plate tests.

demonstrated that they were phenotypically indistinguishable from WT animals with respect to major motor and behavioral parameters; this is why only WT animals were used as controls.

For genotyping, PCR was conducted with primers 5′-ACCTGAGCCCAAGAGACTTCC-3′ (forward) and 5′-GCTACGCTATCAAAGGCCCG-3′ (reverse). The amplicons were cleaved with Mln1 endonuclease, and the digestion products were analyzed. TPH2-KO and WT rats were obtained by intercrossing TPH2-HET parents. A detailed description of the TPH2-KO rat line, including generation, breeding strategy, and genotyping protocol, has been published in (Kaplan et al., 2016; Sysoev et al., 2025).

Experimental design. Figure 1 shows the experimental setups used in the study. All behavioral tests were conducted during the light phase of the circadian cycle (09:00–17:00), with test order counterbalanced across subjects. Kinematic, sensory sensitivity, and grip strength tests were conducted on separate days. To avoid potential interference, sensory sensitivity and grip strength testing always followed the kinematic tests.

Kinematic analysis of locomotion. Gait kinematics were assessed using the MotoRater system (TSE Systems, Germany), which records movement in two views: bottom view for footprint analysis, and side view for joint kinematics. This software allows for the simultaneous acquisition of spatiotemporal parameters and detailed biomechanical data, as well as triangulation-based knee joint positioning to minimize errors arising from direct placement of skin markers (Bauman, Chang, 2010).

The animals were acclimated to the treadmill for three days, using a darkened shelter as a stimulus. Contrast markers were applied to the joints and bony landmarks once under sedation (medetomidine, i.m., 0.4 mg/kg). Video recording was performed at 100 frames per second, and the position of the knee joint was determined using triangulation. For analysis, 15 regular locomotor cycles per rat were selected, excluding recordings with stops or disruptions in cyclicity, which minimized the influence of supraspinal control and allowed focus on the spinal mechanisms of motor pattern generation.

The following kinematic parameters were analyzed: joint angles, angular velocities, and accelerations in three phases of locomotion; spatial characteristics: step height, position of the hip joint and iliac crest, minimum and maximum height of the tail tip; temporal parameters: duration of the stance and swing phases, total locomotor cycle duration; coordination indices: limb retraction and protraction, overlap of stance phases (coupling); spatiotemporal characteristics: step length and width, distance between opposite limbs during stance, average locomotion speed; angles, angular velocities, and angular accelerations (calculated automatically using the MotoRater

system software). Maximum angular velocities and accelerations were considered within a single locomotor cycle. When defining the phases, point E3 for the limb under study was determined as the moment of paw off (toe-off), point F as the midpoint of the swing phase (maximum flexion), and point E1 as the moment of paw contact (touchdown). A complete list of the calculated kinematic parameters is provided in Table S1[1]. Retraction was defined as the distance from the projection of the iliac crest point onto the X-axis to the projection of the metatarsophalangeal joint point onto the X-axis at locomotor point E3; thus, this parameter reflects the maximum backward extension of the limb before the onset of the swing phase. Protraction was determined in a manner analogous to retraction, but at locomotor point E1, describing the maximum forward extension of the limb at the beginning of the stance phase.

The correlation coefficients (parameters 59–88, Table S1) between joint heights reflect intralimb coordination. They were calculated as follows: for each joint, Y-coordinates were determined as a function of time and distance traveled; each set of Y-coordinates was assigned to a phase: stance (from locomotor point E1 to point E3), flexion (from point E3 to point F), or extension (from point F to point E1). Subsequently, correlations between the Y-values of all joint pairs within a single phase were calculated for individual animals.

Analysis of motor coordination function in the Horizontal Ladder test. Motor coordination function was assessed using the MotoRater system (TSE Systems, Germany) with a horizontal ladder (length 170 cm, rung spacing 1 cm), which allowed recording of animal movement from the ventral and lateral views. To evaluate sensorimotor deficit in this test, the total number of steps, errors (misplacements when placing the limb on the rung), and slips of the animal off the rungs were recorded. For each rat, the degree of sensorimotor deficit (SD) was calculated using the equation:

$$SD = (\text{n errors} + 0.5\,(\text{n slips})/\text{total number of steps}) \times 100\ \%.$$

Hot/Cold Plate. To assess sensory function (thermal sensitivity), the Hot/Cold Plate test was performed using a Cold/Hot Plate apparatus (Bioseb, Chaville, France). Thermal sensitivity was evaluated using the Hot Plate (+48 °C) and Cold Plate (+4 °C) setups, recording the latency of reflexive paw withdrawal or licking of the hind paws over a 60-second period (Yalcin et al., 2009).

Grip strength was measured using a Grip Strength Meter 303500 dynamometer (TSE Systems, Germany). Forelimb grip strength was assessed using a T-shaped bar separately for the left paw, right paw, and both paws together.

Statistics. Data processing and statistical analysis were performed using GraphPad Prism 9.0.0 software. The normality of quantitative variable distribution was tested using the Shapiro–Wilk W test. For normally distributed quantitative variables, significance of differences was assessed using two-way ANOVA with Šídák's post hoc test. To account for the effect of velocity on the calculated values, ANCOVA was applied. Correlation analysis was performed using Pearson's correlation coefficient. Numerical data presented in the figures are shown as mean ± SEM.

[1] Supplementary Tables S1, S2 and Figures S1, S2 are available at: https://vavilovj-icg.ru/download/pict-2026-30/appx48.pdf

Since more than 80 kinematic characteristics of locomotion were calculated in the present study, and most of them are highly correlated with each other, we used Principal Component Analysis (PCA). This approach reduces data dimensionality by identifying correlated original variables that form new integrative variables called principal components. Previously, this method was successfully used to identify functional clusters of kinematic, kinetic, and myographic characteristics of locomotion, as well as to assess the selective effects of highly selective pharmacological agents, namely agonists and antagonists of monoamine receptors (Musienko et al., 2011).

Results

Kinematic analysis results

PCA of the parameters showed that 29.6 % of the data variance was explained by PC1, which was formed by spatiotemporal characteristics of locomotion (stance and swing phase durations, average speed, coupling) and step length. PC2 values (20.1 % of data variance) were determined by joint correlation values characterizing intralimb coordination. PC3 (13.6 %) was formed by the magnitude of the hip joint angle in phases E3, F, and E1, the metatarsophalangeal joint angle in phase F, the angular velocities and accelerations of the knee and metatarsophalangeal joints, as well as the correlation between the hip and knee joints during paw extension. PC4 (6.9 %) was influenced by the knee joint angle during maximum flexion and extension, the metatarsophalangeal joint angle at touchdown, hip and iliac crest height, and retraction/protraction. The quantitative contribution of each kinematic parameter to the formation of each principal component was expressed as factor loading values (Fig. 2).

PC1 (29.6 % of data variance) was formed primarily by parameters correlating with average locomotion speed (Fig. 2, PC1). Two-way ANOVA showed a significant effect of the genotype ($F_{(1,34)} = 18.64$, $p = 0.001$) and sex ($F_{(1,34)} = 15.17$, $p = 0.004$) with no genotype × sex interaction. As revealed by post hoc analysis, PC1 values were significantly higher in WT female rats than in WT male or TPH2-KO female rats ($p < 0.01$ and $p < 0.0001$, respectively). A statistically significant increase in average locomotion speed was found in WT males and TPH2-KO females compared to WT females ($p < 0.001$ and $p < 0.0001$, respectively, by Šídák's multiple comparisons test). Also, TPH2-KO males showed an increase in this parameter compared both to WT males and to TPH2-KO females ($p < 0.01$, by Šídák's multiple comparisons test) (Fig. 3*a*). An increase in locomotor cycle duration was noted in WT females compared to TPH2-KO females ($p < 0.0001$, by Šídák's multiple comparisons test). Stance phase duration increased as well ($p < 0.0001$, by Šídák's multiple comparisons test). Meanwhile, swing phase duration in WT females was statistically significantly higher compared to WT males ($p < 0.05$, by Šídák's multiple comparisons test) but not to TPH2-KO females. The values of all the above-described parameters in WT males were at the same level as in rats from the other study groups. The distribution of values among groups for the described parameters is similar to that for PC1, and it

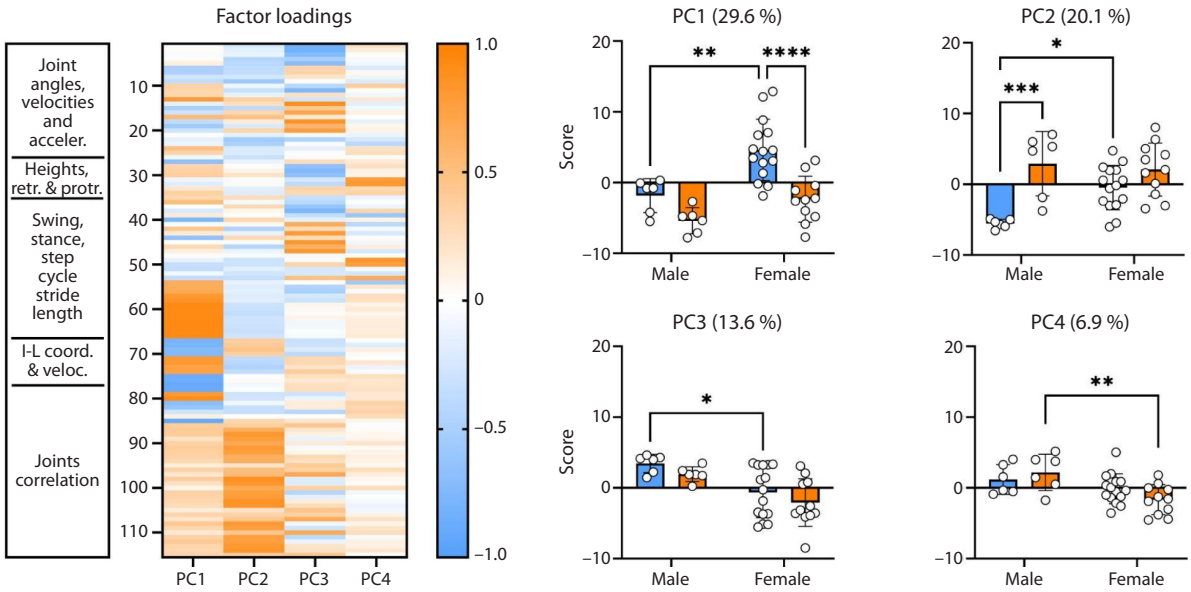


Fig. 2. Factor loadings and values of the four principal components in WT and TPH2-KO rats.

l-L coord. = interlimb coordination. Blue bars represent wild-type (WT) rats, orange bars represent TPH2-KO rats. Male and female groups are indicated on the X-axis. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ indicate statistically significant differences according to Šídák's post hoc test.

points to an inverse relationship between its magnitude and average locomotion speed.

PC2 (20.1 % of data variance) was formed primarily by correlation coefficients between joint heights during the stance and swing phases (the swing phase was divided into two subphases – flexion and extension – with correlations calculated separately for each) (Fig. 2, PC2). We observed a significant effect of the genotype ($F_{(1,34)} = 21.96$, $p < 0.0001$) and genotype × sex interaction ($F_{(1,34)} = 6.30$, $p < 0.0170$) but without role of sex. A decrease in the magnitude of this component was shown in WT males compared to TPH2-KO males and WT females ($p < 0.001$ and $p < 0.05$, by Šídák's multiple comparisons test). A statistically significant decrease in the correlation coefficient between the iliac crest and ankle joint during the extension phase was shown in WT males compared to TPH2-KO males and WT females ($p < 0.001$ and $p < 0.01$, respectively, by Šídák's multiple comparisons test) (Fig. 3*b*). An entirely analogous pattern was observed for the correlation coefficient between the knee and ankle joint heights during the extension phase ($p < 0.01$ and $p < 0.05$, respectively, by Šídák's multiple comparisons test). A decrease in the correlation between the knee and metatarsophalangeal joints during the extension phase was also found in WT males compared to the same groups ($p < 0.05$, by Šídák's multiple comparisons test). Meanwhile, the correlation coefficient between the knee and metatarsophalangeal joints during the stance phase was statistically significantly lower in WT males only compared to TPH2-KO males ($p < 0.01$).

The main contribution to PC3 values (13.6 % of data variance) came from certain joint angle, angular velocity, and angular acceleration parameters (Fig. 2, PC3). We found a significant role of sex ($F_{(1,34)} = 14.93$, $p < 0.0005$) with no effect of genotype or genotype × sex interaction. A statistically significant decrease in this parameter was shown in WT females compared to WT males ($p < 0.05$ by Šídák's multiple comparisons test). For individual parameters contributing to this component (Fig. 3*c*), the following statistically significant differences were identified among the four study groups: the hip joint angle at point E3 was statistically significantly higher in TPH2-KO females compared to TPH2-KO males ($p < 0.05$, by Šídák's multiple comparisons test); at point F, this joint angle was also statistically significantly higher in TPH2-KO females compared to TPH2-KO males ($p < 0.01$, by Šídák's multiple comparisons test); and at point E1, this angle was statistically significantly higher in females of both groups compared to males with the same genotype ($p < 0.05$ for WT and $p < 0.05$ for TPH2-KO, by Šídák's multiple comparisons test). Meanwhile, for the maximum angular acceleration of the knee joint, which also made a significant contribution to PC3 formation, no differences were found between the groups.

PC4 (6.9 % of data variance) was formed primarily by postural parameters (Fig. 2, PC4). Two-way ANOVA showed a significant effect of sex ($F_{(1,34)} = 11.39$, $p = 0.0019$) without effect of genotype or genotype × sex interaction. This component was statistically significantly lower in TPH2-KO females compared to TPH2-KO males ($p < 0.01$ by Šídák's multiple comparisons test). Individual graphs (Fig. 3*d*) show that the iliac crest height, defined as the maximum within a single locomotor cycle, was statistically significantly higher in TPH2-KO males compared to TPH2-KO females ($p < 0.05$, by Šídák's multiple comparisons test). The hip joint height, defined in the same manner, was lower in both WT females and TPH2-KO females compared to WT males and TPH2-KO males respectively ($p < 0.01$ and $p < 0.001$ for TPH2-KO, by Šídák's multiple comparisons test). A decrease in retraction was found in WT females compared to WT males ($p < 0.0001$, by Šídák's multiple comparisons test) and in TPH2-KO fe-

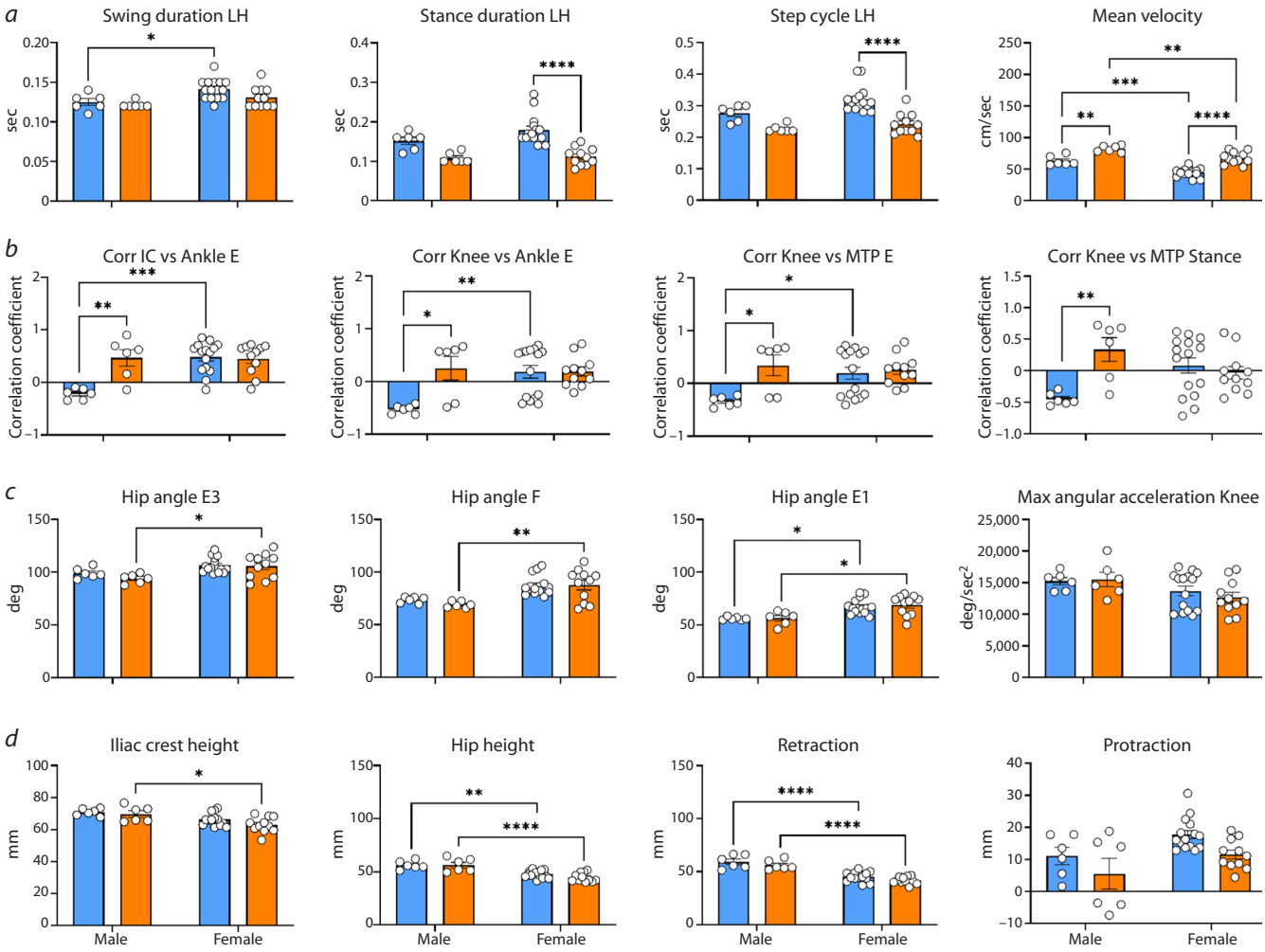


Fig. 3. Comparison of key kinematic parameters of locomotion in WT and TPH2-KO rats forming (*a*) PC1, (*b*) PC2, (*c*) PC3, and (*d*) PC4.
Blue bars represent wild-type (WT) rats, orange bars represent TPH2-KO rats. Male and female groups are indicated on the X-axis. Statistically significant differences according to Šídák's post hoc test: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

males compared to TPH2-KO males ($p < 0.0001$, by Šídák's multiple comparisons test). No statistically significant difference between groups was found in protraction. However, it should be noted that several negative values were present in TPH2-KO males, which is quite atypical for protraction in healthy animals and is likely to be related to their high locomotion speed.

Two-way ANOVA showed a significant effect of sex of the animals on their body weight ($F_{(1,32)} = 402.4$, $p < 0.0001$), while no effect of genotype or genotype × sex interaction on this parameter was observed (Fig. 4). The body weight of WT females was statistically significantly lower than that of WT males ($p < 0.0001$, by Šídák's multiple comparisons test). The body weight of TPH2-KO females was also significantly lower than that of males with the same genotype ($p < 0.0001$, by Šídák's multiple comparisons test).

It was hypothesized that the mass and size of the animals might also influence other locomotion characteristics, such as joint angle magnitudes or postural parameters. Additionally, for each rat, the bone lengths of the left hind limb were calculated, determined as the average lever lengths obtained from kinematic marker placement on the animals. To calculate this group of parameters, we analyzed frames that contained the kinematic skeleton and were included in the processing of the main parameter set. The following conventional lengths were determined: pelvic bone length (from the iliac crest to the hip joint), femur length (from the hip joint to the knee joint), tibia length (from the knee joint to the ankle joint), foot length (from the ankle joint to the metatarsophalangeal joint, excluding the toes), and toe length (from the metatarsophalangeal joint to the distal phalanx). This group of parameters reflects the individual sizes of the animals, which formed the basis for the locomotion characteristics calculated and described above.

Two-way ANOVA revealed a significant main effect of sex on pelvic length ($F_{(1,34)} = 38.07$, $p < 0.0001$) (Fig. 4). WT females had a shorter pelvis than WT males ($p < 0.01$, Šídák's test), and TPH2-KO females showed a reduced pelvic length compared to males of both genotypes ($p < 0.01$ vs. WT males and $p < 0.05$ vs. TPH2-KO males). For femur length, ANOVA demonstrated a highly significant effect of sex ($F_{(1,34)} = 130.4$, $p < 0.0001$). The femur length in WT females was shorter than in either WT males ($p < 0.01$) or TPH2-KO males ($p < 0.05$),

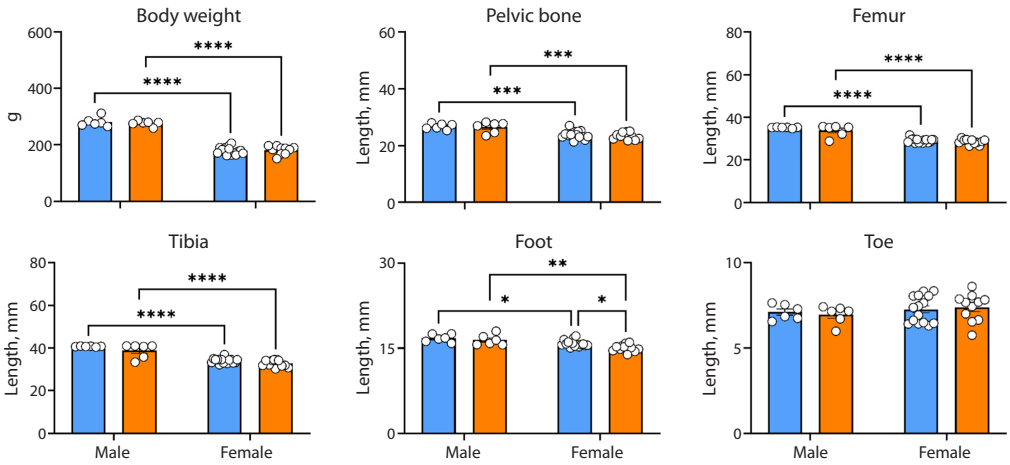


Fig. 4. Body weight and bone lengths of the left hind limb (calculated based on distances between kinematic markers) in WT and TPH2-KO rats.

Blue bars represent wild-type (WT) rats, orange bars represent TPH2-KO rats. Male and female groups are indicated on the X-axis. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ indicate statistically significant differences according to Šídák's post hoc test.

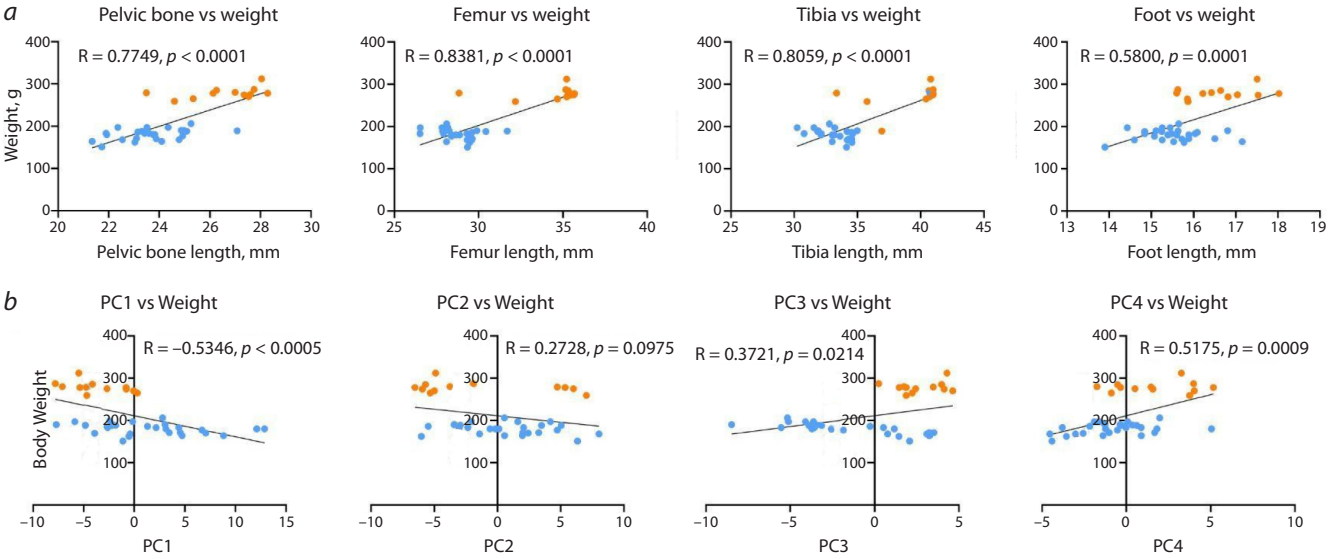


Fig. 5. *a*, Correlations between left hind limb bone lengths (calculated based on distances between kinematic markers) and body weight of the tested rats. *b*, Correlations between animal body weight and PC1–PC4 values.

Blue indicates female rats; orange, male rats.

and the same pattern was observed in TPH2-KO females, whose femurs were shorter than those of males of either genotype ($p < 0.01$ for both comparisons). Analysis of tibia length revealed significant effects of sex ($F_{(1,34)} = 110.2$, $p < 0.0001$) and genotype ($F_{(1,34)} = 6.807$, $p = 0.0134$). WT females had shorter tibias than males of the same genotype ($p < 0.05$), while TPH2-KO females displayed even more pronounced differences, with tibia length significantly reduced compared to either WT males ($p < 0.001$) or TPH2-KO males ($p < 0.01$). With respect to foot size excluding toe length, ANOVA showed significant effects of sex ($F_{(1,34)} = 23.77$, $p < 0.0001$) and genotype ($F_{(1,34)} = 5.568$, $p = 0.0242$). Foot size in WT females did not differ from that of males, whereas TPH2-KO females had smaller feet compared to males of either genotype ($p < 0.001$ vs. WT males and $p < 0.05$ vs. TPH2-KO males). Finally, the calculated toe lengths were similar across males and females of both phenotypes, which may reflect the relatively small magnitude of this measurement in millimeters and the potential for error associated with the resolution of the video recordings.

We calculated correlations between animal body weight and the lengths of the left hind limb bones, as well as between body weight and the principal components PC1–PC4 derived from the main locomotor data (Fig. 5). A high statistically significant correlation was found between the calculated lengths of the pelvic bone (R = 0.7749), femur (R = 0.8381), and tibia (R = 0.8059) and animal weight ($p < 0.0001$). Foot length in this case correlated with rat weight to a lesser extent, but also showed high statistical significance (R = 0.5800, $p = 0.0001$). The results of these calculations confirm that the placement of kinematic markers on the animals was performed satisfactorily

and reflected a direct relationship between the dimensions of the kinematic skeleton and the body weight and individual body size of the rats.

Correlations between animal weight and the four principal components were calculated. PC1 was found to correlate statistically significantly with rat weight (R = −0.5346, $p < 0.0005$). PC4 also showed a high degree of correlation with animal weight (R = 0.5175, $p = 0.0009$). PC3, albeit to a much lesser extent, also correlated with body weight (R = 0.3721, $p = 0.0214$). Meanwhile, no correlation was observed between PC2, which reflected intralimb coordination, and animal body weight (R = 0.2728, $p = 0.0975$). The absence of a correlation in this case may be attributed to the fact that the results of intralimb coordination calculation did not depend on the actual body size of the animals, but rather reflected only the relationship between the trajectories of joint displacement along the Y-axis (height) over a specific time interval (X-axis) associated with the phase of the locomotor cycle.

The complete list of calculated kinematic parameters is presented in Figure S1, and the two-way ANOVA results for the main kinematic parameters are presented in Table S2.

Assessment of motor coordination function in the Horizontal Ladder test

Two-way ANOVA showed a significant effect of genotype on the sensorimotor deficit of forelimb in the Horizontal Ladder test ($F_{(1,43)} = 10.30$, $p = 0.0025$), while no effect of sex or genotype × sex interaction on this parameter was observed. No statistically significant difference was found between TPH2-KO and TPH2 WT by Šídák's post hoc test (Fig. 6). In the TPH2-KO animals, several values exceeding the normal range of sensorimotor deficit were recorded, which may be associated with higher locomotion speed leading to the occurrence of errors.

Assessment of sensory function in the Hot/Cold Plate test

Two-way ANOVA showed a significant effect of sex on pain response duration in hot plate ($F_{(1,42)} = 7.100$, $p = 0.0109$), while no effect of genotype or genotype × sex interaction on this parameter was observed. In assessing pain sensitivity to high (+48 °C) and low (+4 °C) temperatures, no statistically significant difference was found between TPH2-KO and TPH2 WT by Šídák's post hoc test. The TPH2-KO animals exhibited signs of pain response – paw withdrawal or licking – within the same time interval from the start of testing as the WT animal (Fig. 7).

Grip Strength test results

Grip strength assessment revealed no difference between TPH2-KO and WT rats within the same sex, although sex-dependent differences were observed across all measures (Fig. 8). For the right forelimb, two-way ANOVA demonstrated a significant main effect of sex ($F_{(1,20)} = 26.57$, $p < 0.0001$) but no effect of genotype. Right forelimb grip strength did not differ between males of the two genotypes, nor between females of the two genotypes. However, WT males showed a greater strength than WT females ($p < 0.01$) or TPH2-KO females ($p < 0.05$). Similarly, TPH2-KO males outperformed WT females ($p < 0.01$) and TPH2-KO females ($p < 0.05$).

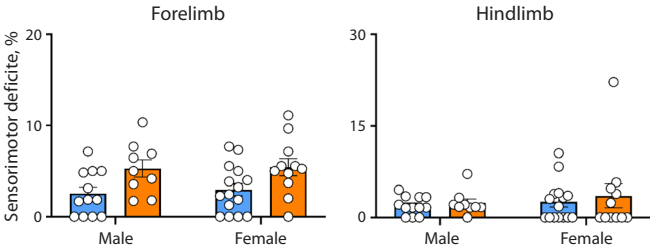


Fig. 6. Results of the assessment of motor coordination function of the hind and forelimbs in rats using the Horizontal Ladder test.

Blue bars represent wild-type (WT) rats, orange bars represent TPH2-KO rats. Male and female groups are indicated on the X-axis.

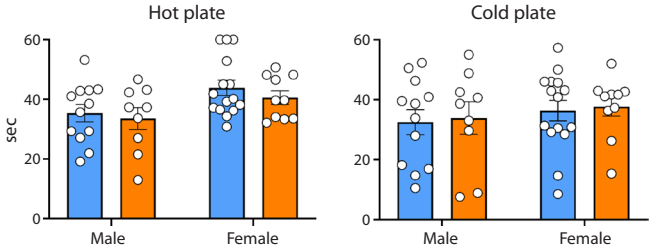


Fig. 7. Pain response latency in the Hot/Cold Plate test.

Blue bars represent wild-type (WT) rats, orange bars represent TPH2-KO rats. Male and female groups are indicated on the X-axis.

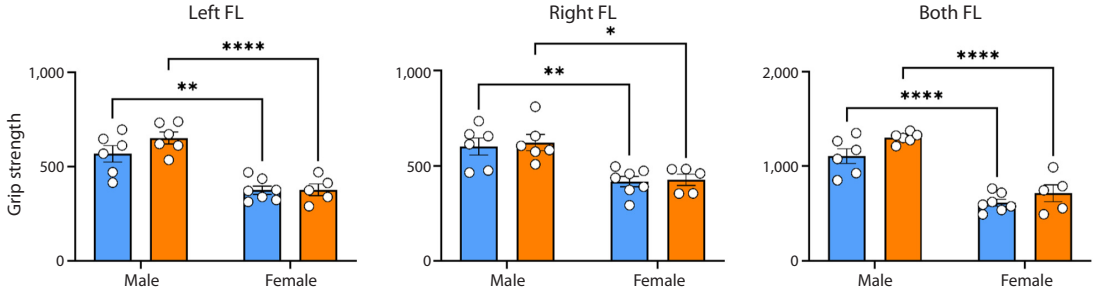


Fig. 8. Grip strength of the left, right, and both forelimbs in WT and TPH2-KO males and females.

Blue bars represent wild-type (WT) rats, orange bars represent TPH2-KO rats. Male and female groups are indicated on the X-axis. * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$ according to Šídák's post hoc test.

Left forelimb grip strength analysis also revealed a significant effect of sex ($F_{(1,20)} = 50.62$, $p < 0.0001$) and no genotype effect. Again, no differences were found between males of different genotypes or between females of different genotypes. WT males had higher left forelimb grip strength than WT females ($p < 0.01$) or TPH2-KO females ($p < 0.01$), while TPH2-KO males showed greater strength than WT females ($p < 0.0001$) or TPH2-KO females ($p < 0.0001$).

Bilateral grip strength showed a significant main effect of sex ($F_{(1,20)} = 83.02$, $p < 0.0001$), along with a significant effect of genotype ($F_{(1,20)} = 6.172$, $p = 0.0220$). No within-sex differences between the genotypes were detected. Bilateral grip strength was higher in males of both genotypes compared to females of both genotypes ($p < 0.01$ to $p < 0.0001$).

Discussion

TPH2 knockout does not cause major sensorimotor deficits, even though serotonin is crucial for developing sensorimotor control structures

Studies of behavioral phenotypes in TPH2-KO animals have primarily focused on identifying emotional, anxiety-related, and cognitive changes; however, these model organisms may also be relevant for sensorimotor research (Meng et al., 2022; Alonso et al., 2024). This assumption is based on the key role of serotonin in the neural control of sensorimotor functions (Ghosh, Pearse, 2015), as well as on the pronounced behavioral alterations observed in TPH2-KO animals (Meng et al., 2022). Central serotonin deficiency exerts a complex disorganizing influence on the development and function of the entire sensorimotor system, as it acts both as a critical trophic factor during brain development (Di Pino et al., 2004) and as a key neuromodulator in adulthood (Kawashima, 2018). The data from the present study demonstrate changes in certain locomotor characteristics in TPH2-KO animals.

The most pronounced changes include a reduction in swing phase and stance phase duration of the left hind limb compared to WT control animals. Overall, the observed increase in locomotion speed in TPH2-KO rats indicates a disruption in motor rhythm regulation, highlighting the role of serotonin as a regulator of dynamic movement parameters. The serotonergic system modulates motor control through complex interactions with key structures (basal ganglia, the cerebellum, and the spinal cord), with its effects being multidirectional and dependent on receptor type, neurotransmitter concentration, temporal activity patterns, and behavioral context (Lesch et al., 1996; Sachenko, Khorevin, 2001; Kawashima, 2018). This system integrates diverse information and appears to employ common computational principles (e. g., activity accumulation) to modulate movements and other functions. It is known that blockade of serotonin receptors or inhibition of serotonin synthesis in rodents often leads to gait impairments. A study on *Tph2*-knockout mice has shown that serotonin deficiency specifically disrupts inhibitory control over actions without causing general hyperactivity (Angoa-Pérez et al., 2012). This behavior manifests itself as pathological motor stereotypies (compulsive burying of familiar and unfamiliar objects) and dysregulation of motor impulsivity, reflected in an inability to suppress responses under potential threat conditions (Novelty Suppressed Feeding test and Dark/Light Emergence Test). Apparently, under normal conditions, serotonergic projections to spinal networks and motoneurons exert a modulatory, often inhibitory influence, and chronic deficiency leads to reduced inhibitory control and increased excitability of motor networks. This is consistent with literature data: increased spontaneous motor activity following damage to the raphe nuclei (Sachenko, Khorevin, 2001), and hyperlocomotion induced by amphetamine administration in TPH2-KO mice (Carli et al., 2015). Furthermore, serotonergic system dysfunctions have been linked to specific sensory and motor phenotypes in ADHD (impulsivity and motor restlessness) (Oades, 2008) and autism (stereotypies and sensory hyper-/hyposensitivity) (Muller et al., 2016).

Figure 9 shows the trajectories of the left hind limb in XY space over two locomotor cycles, displaying displacement curves for each anatomical structure and stick diagrams at

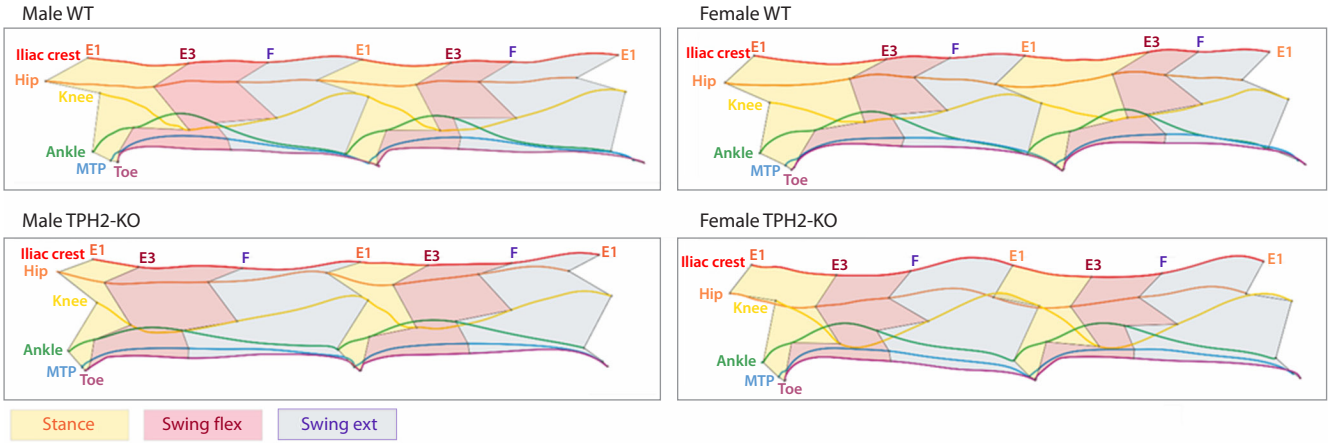


Fig. 9. Representative images of joint trajectories in space over two locomotor cycles. Graphs are shown for individual rats from each group.

Anatomical landmarks: red = iliac crest; orange = hip joint; yellow = knee joint; green = ankle joint; blue = metatarsophalangeal joint; purple = distal phalanx of the fifth toe. Phase designations: yellow = stance; red = flexion; blue = extension.

critical locomotor points that divide the full cycle into three phases. The data obtained in this manner formed the basis for calculating intralimb coordination. The curves formed by joint displacements exhibit different patterns and differ among all four representative rats. While differences between WT rats of both sexes are minimal, in TPH2-KO animals a similar pattern can be identified in both cases: the slope of the curve representing the ankle joint during the extension phase (end of the locomotor cycle) is visually smaller in both TPH2-KO males and females compared to WT rats, which is a direct consequence of increased locomotion speed. Other differences in the presented patterns may be related to individual locomotor characteristics of each representative animal.

Increased locomotion speed is one of the key phenotypic characteristics of TPH2-KO rats. To assess the contribution of speed to the observed kinematic differences, we performed an analysis of covariance (ANCOVA) with locomotion speed as the covariate. The ANCOVA results demonstrated that, after adjusting for speed, statistically significant differences between TPH2-KO and WT animals disappeared for the following parameters: swing and stance phase durations; hip joint angles at points E3, F, and E1; retraction magnitude; heights of the hip joint and iliac crest; and several intralimb coordination indices (see Fig. S2). The only parameter for which a statistically significant difference between genotypes persisted after adjusting for speed was the correlation between iliac crest and ankle joint trajectories during the extension phase (Corr Iliac Crest vs Ankle E) in males.

Critically, there were no differences between TPH2-KO rats and WT animals in intralimb coordination parameters (comprising PC2), interlimb coordination parameters, or postural function. This indicates that the basic spinal and supraspinal pathways generating and coordinating stepping movements remain intact. The results of the Hot/Cold Plate and Grip Strength tests in the present study serve as additional evidence for the "preservation" of the sensorimotor system in TPH2-KO rats. Yet it is known that central serotonin deficiency critically influences the development of neural structures and functions involved in sensorimotor control. As a developmental regulator, serotonin influences neurogenesis, synaptogenesis, and dendrite formation, and also participates in the maturation of brain structures that enable sensorimotor integration (Sodhi, Sanders-Bush, 2004; Shiga et al., 2006). The growth retardation and impaired somatosensory reflexes observed in TPH2-deficient pups further confirm the involvement of serotonin in sensorimotor development processes (Wang et al., 2024). At the same time, congenital serotonin deficiency appears to trigger powerful compensatory and adaptive mechanisms during nervous system development. The associated changes in locomotion kinematic characteristics point to a reorganization of central motor control mechanisms under serotonin deficiency. This is supported by data showing hyperinnervation of certain areas (hippocampus, nucleus accumbens) and increased BDNF expression in TPH2-KO mice; moreover, despite serious growth and survival issues, no gross anatomical changes were detected in the brains of TPH2-KO mice (Migliarini et al., 2013).

The presented results of principal component analysis (PC3 and PC4) reveal complex and statistically significant differences in gait kinematics between females and males. These differences affect both dynamic movement parameters (angles, velocities, accelerations) and postural characteristics. PC3 primarily reflects features of joint angular dynamics, particularly of the hip joint. The key finding is the statistically significantly lower PC3 values in females (both WT and TPH2-KO) compared to WT males, indicating a fundamental difference in movement strategy related to sex and animal size. The hip joint angle in specific gait phases (points E3 and F) is systematically larger in females compared to males (most pronounced when compared to TPH2-KO males). This may indicate more pronounced hip extension in females at the end of the stance phase and the beginning of the swing phase.

PC4 primarily describes postural features. Here, the pattern is more complex, and it demonstrates an interaction effect between sex and genotype. A statistically significant decrease in PC4 is observed only in one group – TPH2-KO females compared to TPH2-KO males – indicating that serotonin deficiency (due to *Tph2* gene knockout) differentially modifies movement posture in males and females. Females of both groups exhibit reduced hip joint height during walking compared to WT males. This may be a direct consequence of anatomical differences or gait features aimed at increasing stability. Females of both groups showed reduced retraction compared to males, suggesting that in females, the limb is less extended backward relative to the body at the end of push-off. This feature may correlate with shorter step length.

It should be noted that the existing literature contains limited data for direct comparative assessment of the obtained kinematic parameters. This situation is due to the predominance of studies conducted on males, creating a significant gap in understanding the specific features of locomotor activity in females. In this context, the presented results make an important contribution to describing the normal physiological patterns of locomotion in female laboratory rats.

The straightest explanation for the observed kinematic differences is anatomical features, particularly sexual dimorphism of the pelvic girdle. Morphological studies confirm that the female-specific pelvic shape, determined by the reproductive function, is formed during postnatal ontogeny under the influence of hormonal factors (Berdnikovs et al., 2007). Furthermore, accumulating evidence suggests that males and females may employ different neurobiological strategies to organize outwardly similar movements, and these differences are established in the CNS under hormonal influence during critical developmental periods (Field, Pellis, 2008).

Sex-dependent coordination patterns in rats, which do not require conscious control, may be shaped during ontogeny under the influence of sex hormones, for example, through modulation of the basal ganglia dopaminergic system, which is critical for movement automation (Kompoliti, 1999; Colzato, Hommel, 2014). Hormonal regulation itself may be governed by other neurotransmitter systems, and to test the hypothesis of cascade interactions between neurotransmitter systems regulating motor function, it is necessary to determine how

5-HT deficiency, which plays a key role in modulating neuroendocrine axes, affects sex hormone levels and metabolism during critical periods of sensorimotor brain development (Rybaczyk et al., 2005).

It is noteworthy that negative protraction values were recorded in some TPH2-KO males. This parameter reflects paw position behind the iliac crest projection at stance onset and deviates from protraction values typical of intact rats. Such paw placement at initial contact is explained by the tendency of rats to position their hind paws further back relative to the body at touchdown during high-speed locomotion (Neckel, 2015), providing more stable body support. In TPH2-KO rats, the preference for high locomotion speed is a characteristic of this genetic line, particularly pronounced in males.

Limitations and future directions

Using only one age group limits the findings, as the impact of *Tph2* knockout on sensorimotor function and neuroplasticity may be age-dependent (Ririe et al., 2003). Therefore, some effects of TPH2-KO may have remained undetected, and future studies should include multiple age groups. We also acknowledge as a limitation the age difference between males (10–12 months) and females (12–16 months), as well as the within-group scatter in females up to four months. Age-related changes in motor and serotonergic functions cannot be ruled out. However, if older age systematically had impaired gait, WT females would have performed worse than WT males, which we did not observe. Nevertheless, future studies should standardize age to better demarcate genotype and sex effects.

The sensory assessment was limited to thermal sensitivity tests. Although the Hot/Cold Plate tests are informative for evaluating thermonociception, they do not characterize other somatosensory modalities such as mechanical or tactile sensitivity. Use of a broader battery of sensory and nociceptive tests (von Frey filaments, Hargreaves test (Cheah et al., 2017), Cold Plantar Assay (Brenner et al., 2015), and Randall–Selitto test) could characterize the sensorimotor phenotype of TPH2-KO rats in more detail and reveal subtle sensory processing deficits that may remain undetected when using only thermal tests.

Conclusions

The present study reveals that congenital central serotonin deficiency in TPH2-KO rats does not impair basic motor coordination or sensory functions. However, principal component analysis demonstrates that the main phenotypic manifestation is a statistically significant increase in locomotion speed, which mediates complex changes in gait kinematics, including modulation of intralimb coordination and postural parameters partially dependent on body weight and size. Thus, serotonin plays a fine modulatory role in controlling speed characteristics and associated spatiotemporal movement patterns without affecting the basic organization of sensorimotor functions. Rats with *Tph2* gene knockout are particularly valuable for studying the role of serotonin in sensorimotor recovery after spinal cord injury due to the preservation of basic motor function against the background of systemic serotonin deficiency.

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