

Tph2 deficiency shapes neurodevelopmental behavioral trajectories through interactions with the maternal environment, sex, and the gut microbiome

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Received: 25 February 2026 / Revised: 16 July 2026 / Accepted: 28 July 2026

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Abstract

Serotonin (5-HT) is critical for neurodevelopment, influencing brain maturation, emotional regulation, and social behavior. Tryptophan hydroxylase 2 (TPH2), the rate-limiting enzyme for central 5-HT synthesis, causes lifelong 5-HT depletion when disrupted. While early-life 5-HT perturbations are linked to neuropsychiatric vulnerability, it remains unclear how lifelong depletion shapes behavioral trajectories across development, or how sex and postnatal environment influence these effects. Here, we provide a longitudinal characterization of *Tph2*-deficient male and female rats, assessing reflex development, sensorimotor coordination, anxiety-like behavior, recognition memory, social interaction, and aggression across the first two months of life. To investigate environmental and microbial modulators, we quantified maternal care (e.g., licking/grooming, nursing, nest proximity) through daily observations during the first postnatal week and profiled adult gut microbiota via 16S rRNA sequencing. The association between maternal care and behavioral outcomes was assessed to identify environmental modulators of the *Tph2* phenotype, while principal component analysis (PCA) visualized genotype- and sex-specific microbial clustering. *Tph2*^{-/-} rats exhibited delayed sensorimotor maturation, altered emotional regulation, reduced object-directed exploration and sociability, and increased aggression, particularly in males. Sensorimotor and exploratory outcomes were positively associated with maternal care, reinforcing its role in shaping behavioral trajectories. Microbiota analysis revealed genotype- and sex-dependent alterations, with *Tph2*^{+/-} males showing the most pronounced shifts, including increased microbial richness and taxonomic divergence. Together, our findings demonstrate that 5-HT depletion disrupts behavioral neurodevelopmental trajectories via interactions between genetic, environmental, and ecological factors, highlighting the importance of accounting for sex, environment, and developmental timing in translational research.

Introduction

Beyond its role as a neurotransmitter, serotonin (5-HT) acts as a trophic factor during brain development, influencing neurogenesis, differentiation, axon myelination, and synaptogenesis [1–4]. Disruption of early 5-HT signaling is implicated in neuropsychiatric disorders [5–7], including schizophrenia [8], autism spectrum disorder (ASD) [9–11],

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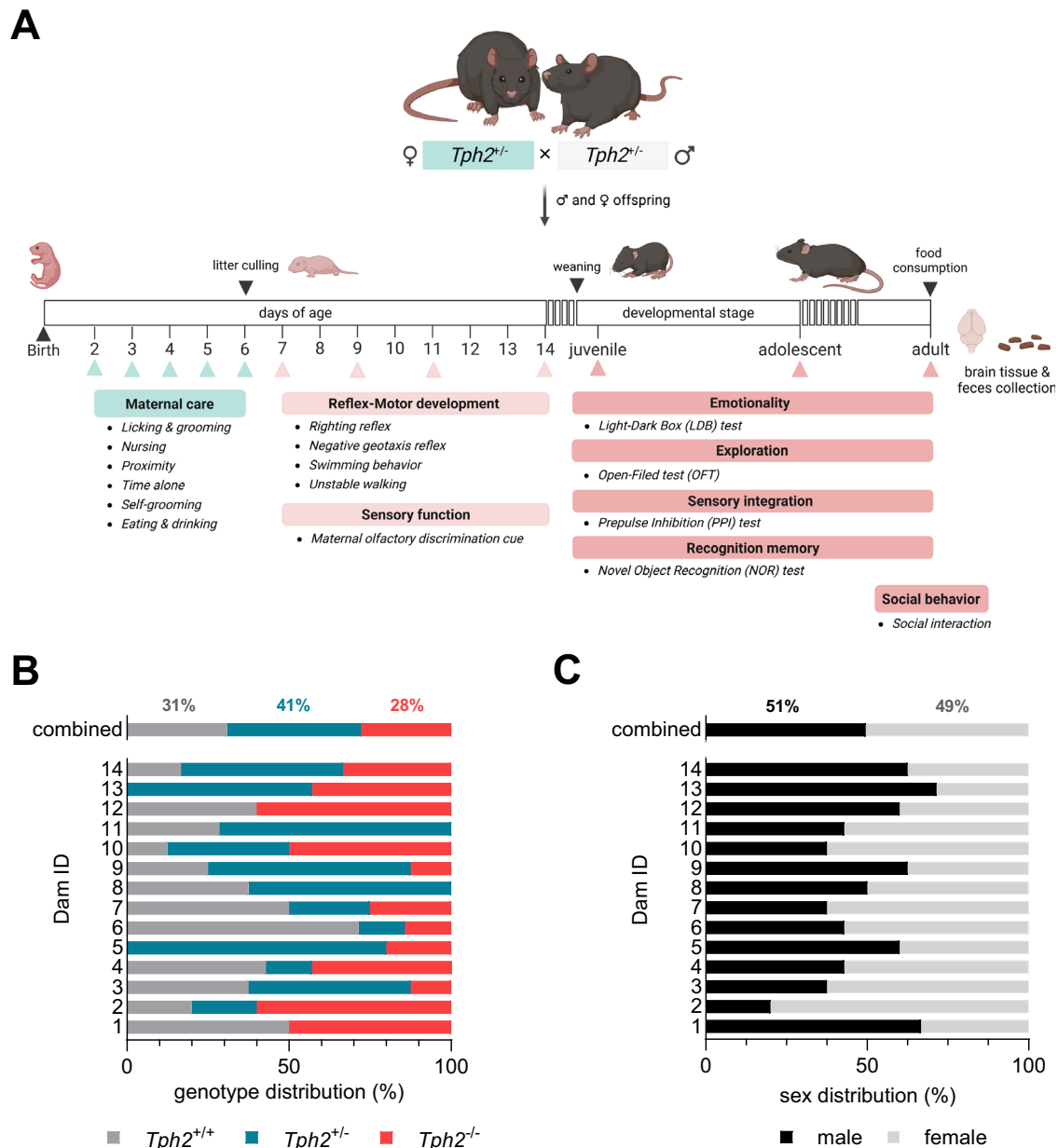


Fig. 1 Overview of the study design, experimental timeline, and offspring genotype and sex distribution. **A** Experimental timeline. Breeder pairs heterozygous for TPH2 (*Tph2^{+/-}*) were set up to generate male and female wild-type (*Tph2^{+/+}*), heterozygous (*Tph2^{+/-}*), and homozygous (*Tph2^{-/-}*) offspring. Offspring included $n = 48$ males (13 *Tph2^{+/+}*, 20 *Tph2^{+/-}*, 15 *Tph2^{-/-}*), $n = 49$ females (17 *Tph2^{+/+}*, 20 *Tph2^{+/-}*, 12 *Tph2^{-/-}*), from a total of 14 litters. Maternal care was assessed between postnatal day (P)2 and P6, including the frequencies of licking and grooming, nursing, proximity of the litter, time spent alone, self-grooming, or eating and drinking. Litters were culled at P6. Reflex development (righting, negative geotaxis), swimming behavior (position and direction), unstable walking, and maternal olfactory discrimination were evaluated at P7, P9, P11, and P14. Pups were weaned at P21. From P21 onward, animals were subjected to a 5-day behavioral battery at juvenile (P21-P25), adolescent (P35-P39), and adult (P70-P74) stages. Tests were conducted in a fixed sequence: light-dark box (LDB; emotionality), open field test (OFT; exploration), prepulse inhibition (PPI; sensory integration), and novel object recognition (NOR; recognition memory), with 24-h intervals between assays. Social behavior was assessed in adulthood. All behavioral assessments were conducted during the light phase (lights on at 07:00, lights off at 18:00) within standardized time windows for all experimental groups. Following the final behavioral assessment, animals were euthanized, and brain tissue and fecal samples were collected. Fecal pellets were used for 16S rRNA gene sequencing. Created with Biorender.com. **B, C** Genotype and sex distribution of experimental rat pups across all 14 litters. *Tph2*, Tryptophan hydroxylase 2.

depression [12], and anxiety [13]. Importantly, 5-HT dysfunction often precedes clinical onset, suggesting a developmental contribution to disease vulnerability. However, it remains unclear how lifelong 5-HT depletion shapes emerging behavioral phenotypes, particularly the extent to which sex and environmental factors interact with these trajectories.

In the central nervous system (CNS), 5-HT synthesis is mediated by tryptophan hydroxylase isoform 2 (TPH2) [14], whereas TPH1 orchestrates most peripheral 5-HT synthesis. Multiple constitutive *Tph2* knockout (*Tph2*^{-/-}) rodent models [15–25] have elucidated the consequences of 5-HT depletion, including neonatal growth retardation [16, 17, 22, 24, 26, 27] and increased mortality [16, 23]. Notably, while *Tph2*^{-/-} neonates show successful nutrient ingestion at birth, growth deficits persist during the first three postnatal weeks [16]. Together, these findings suggest that central 5-HT deficiency induces a lasting alteration in developmental scaling rather than a transient delay, underscoring the importance of longitudinal postnatal characterization.

Tph2^{-/-} animals exhibit elevated aggression [16, 24, 28, 29] and pronounced maternal care deficits, including pup-directed attacks [16, 30]. Emotional and cognitive outcomes remain inconsistent; some studies report paradoxical reductions in anxiety-like behavior in adulthood [25, 28, 29, 31–33], while others find no effect [28]. Because most research has focused on adult stages, the neurobehavioral ontogeny of these phenotypes remains largely unexplored. These discrepancies highlight the need for longitudinal approaches to identify the timing and progression of behavioral alterations in emotional regulation.

Tph2 deficiency may also influence development indirectly through early-life environmental factors. *Tph2*^{-/-} dams display impaired maternal behavior, characterized by delayed pup retrieval and impaired nest building, which can shape offspring outcomes independently of pup genotype [16, 30]. Such environmental factors represent a critical yet under-investigated source of variation in functional neurodevelopmental trajectories in 5-HT depletion models.

Beyond central circuits, 5-HT is a key regulator of the gut-brain axis [34]. Enteric 5-HT controls gastrointestinal motility, while the gut microbiota reciprocally modulates host 5-HT synthesis and neurodevelopmental outcomes [34–36]. In humans, altered 5-HT signaling is associated with gastrointestinal comorbidities in neuropsychiatric disorders [37, 38]. Although changes in 5-HT availability can reshape microbial composition [39, 40], the interplay among TPH2 depletion, sex, and the microbiota remains unexplored. Characterizing microbial richness (α -diversity) and taxonomic divergence is therefore essential for defining the systemic impact of lifelong 5-HT deficiency.

Most studies to date focus on adult animals, leaving the ontogeny of behavioral trajectories unclear. However, the 5-HT system undergoes substantial remodeling from postnatal day (P)0 to P21, a window of heightened neuroplasticity and sensitivity to environmental input [41]. Furthermore, while sex differences in 5-HT modulation of stress-responsive circuits are well-documented [42–44], they remain largely unexplored in the context of lifelong TPH2 deficiency.

To address these gaps, we conducted a longitudinal characterization of *Tph2*^{+/+}, *Tph2*^{+/-}, and *Tph2*^{-/-} rats from P7 to P70, assessing sensorimotor development, socio-emotional behavior, and sensorimotor gating. In parallel, we evaluated early-life maternal care and adult gut microbiota as environmental and ecological modulators. By modeling associations between neonatal caregiving and offspring behavioral outcomes, we aimed to identify the environmental contributors to the *Tph2*-deficiency phenotype. Our findings demonstrate that *Tph2*-deficiency disrupts behavioral neurodevelopmental trajectories through sex-specific interactions with maternal care and microbial factors. Together, this integrative framework examines the ontogeny of behavioral dysfunction and offers translational insights into the developmental origins of affective neuropsychiatric disorders.

Materials and methods

Animals, breeding, and housing conditions

Tph2-deficient rats (Dark Agouti) were generated via zinc-finger nuclease technology [23]. Experimental wild-type (*Tph2*^{+/+}), heterozygous (*Tph2*^{+/-}), and homozygous (*Tph2*^{-/-}) animals were bred from *Tph2*^{+/-} pairs (Fig. 1A).

Given that group assignment was predetermined by genetic inheritance, formal randomization was not performed. At postnatal day (P)5–6, following sex determination and high-resolution melting (HRM)-based genotyping (Supplementary Methods; Supplementary Fig. 1A–B), litters were standardized to $n = 8$ pups (balanced for genotype/sex), resulting in 14 litters. The experimental cohort included 48 males (13 *Tph2*^{+/+}, 20 *Tph2*^{+/-}, 15 *Tph2*^{-/-}) and 49 females (17 *Tph2*^{+/+}, 20 *Tph2*^{+/-}, 12 *Tph2*^{-/-}; Fig. 1B, C). Sample sizes were determined by a priori power calculations (Supplementary Methods). Post-weaning, rats were housed in sex- and genotype-matched groups of 2–3 in Macrolon® type 3 cages (21 ± 1 °C, 50–60% humidity, 12-h light-dark cycle) with ad libitum food (ssniff, Germany; Supplementary Table 1) and water. Longitudinal assessments occurred at the neonatal (P7–P18), juvenile (P21–P25), adolescent (P35–P39), and adult (P70–P74) stages (Fig. 1A). Neonatal milestones, including swimming behavior, unstable walking, and maternal olfactory discrimination, were assessed during P7–P14 (Supplementary Methods). From P21 onward, animals were subjected to a 5-day behavioral battery conducted in a fixed sequence: light-dark box (LDB; emotionality), open field test (OFT; exploration), prepulse inhibition (PPI; sensory integration), and novel object recognition (NOR; recognition memory), with 24-h inter-test intervals. Behavioral testing was conducted during the light phase (lights on at 07:00, lights off at 18:00) at standardized time windows across experimental groups. The testing sequence was designed to prioritize anxiety-related assays under conditions of maximal novelty (LDB, OFT), followed by sensorimotor gating (PPI) and cognitive (NOR) assessments. Social behavior was assessed in adulthood. All animals were tested under identical circadian and experimental conditions. Data were collected blindly; room specifications and sanitization protocols are detailed in the Supplementary Methods. Following the final assessment in adulthood, food intake was recorded for five days (Supplementary Methods). Rats were subsequently anesthetized (pentobarbital, 60 mg/mL, via intraperitoneal injection) for fecal collection and transcardial perfusion with 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS, pH 7.4). All procedures were performed under project license AVD10300 2020 1142 in full compliance with the Dutch Animal Testing Act.

Maternal care

Maternal behavior was assessed between P2–P6 (Fig. 1A) under a 12-h light-dark cycle, with lights on at 07:00 and off at 18:00. Direct observations were conducted during four daily 60-min sessions: 08:00 (early light phase), 12:00 (mid light phase), 16:00 (late light phase), and 19:30 (early dark phase). Dams' behavior was scored every 3 min (420 total observations/dam) as present/absent for: (i) licking/grooming, (ii) nursing more than half of the litter (passive, low-back, and arched-back postures), (iii) litter proximity (contact without licking and grooming or nursing), (iv) time off-nest, (v) self-grooming, and (vi) eating/drinking.

Morphological and reflex development

Body weight was measured every two days (P7–P21) and weekly thereafter (Fig. 1A). Growth milestones and neonatal reflexes were assessed as previously described [45]. Eye-opening was monitored daily from P7, with the number of eyes opened recorded (0–2). Postural control (righting reflex) and vestibular function and spatial orientation (negative geotaxis; 40° incline) were evaluated at P7, P9, P11, and P14, recording the latency (up to 60 s) to return to a prone position or rotate 90°, respectively.

Light-Dark Box (LDB)

Anxiety-like behavior was assessed during the juvenile, adolescent, and adult stages (Fig. 1A) using a two-chambered apparatus (bright: 28.2 × 28.2 cm, 12.1 lux; dark: 30 × 30 cm). Rats were placed in the dark chamber with 5 min of free access to the light compartment. Parameters recorded using EthoVision XT (v10.1, Noldus, Wageningen, the Netherlands) included time spent in the light chamber, velocity, total distance, and entry frequency (full and head-only). Elevated plus maze testing at P21 was excluded due to consistent, genotype-independent escape behavior.

Open Field Test (OFT)

Exploratory behavior and locomotor activity were assessed during the juvenile, adolescent, and adult stages (Fig. 1A). Rats were placed in a square arena (50 × 50 × 50 cm) facing the outermost corner and allowed to explore for 10 min. Tracking using EthoVision XT defined the center zone as the innermost 16.7 × 16.7 cm area (12.1 lux). Evaluated parameters included total distance, velocity, and the percentage of time spent and distance traveled in the center.

Novel Object Recognition (NOR)

Intermediate-term memory and exploration were assessed during the juvenile, adolescent, and adult stages (Fig. 1A) in a 50 × 50 × 50 cm arena. In the first trial, rats explored two distinct objects (5 cm from the walls) for 7 min. After a 4-h interval, the outermost object was replaced by a novel object for a 7-min test trial. Object sets were counter-balanced, and different sets were used for each developmental stage. Investigations, defined as sniffing or nose-contact within 2 cm, were manually scored using Observer XT software (v12.5, Noldus). The novel object exploration ratio was calculated as: $\frac{\text{exploration time novel object}}{\text{total exploration time}}$ [46].

Acoustic startle reflex and prepulse inhibition (PPI)

Sensorimotor gating was assessed during the juvenile, adolescent, and adult stages (Fig. 1A) with the SR-LAB startle response system (San Diego Instruments, USA) [47] using age-appropriate Plexiglass cylinders (diameters: juvenility, 3.8 cm; adolescence, 6 cm; adulthood, 8.2 cm). Following a 5-min habituation (70 dB background noise), sessions included: (i) six initial 120 dB startle pulses (20 ms); (ii) twelve blocks of five pseudo-randomized trials (one 120 dB startle, one no-stimulus, three prepulse-startle pairings), and (iii) six final 120 dB pulses. Prepulses (20 ms) were 5, 10, or 15 dB above background, preceding the 120 dB pulse by 100 ms. Intertrial intervals ranged from 10 to 20 s. Startle amplitude was defined as the median response to startle-only trials. PPI was calculated as: $100 - \left(\frac{\text{median startle amplitude on prepulse trials}}{\text{median startle amplitude on startle trials}} \right) \times 100$.

Social interaction

Social behavior was assessed in adulthood (Fig. 1A) following 3-h of isolation. Each rat was placed in a 50 × 50 × 50 cm arena with an unfamiliar, genotype-, sex-, and weight-matched (±10%) conspecific for 15 min. Dyadic behavior was manually scored using Observer XT and categorized as: (i) non-social (self-grooming, exploration, solitary rearing, resting); (ii) affiliative (sniffing, passive chasing, body contact, allogrooming, play fighting); and (iii) agonistic (biting, kicking, aggressive grooming, active chasing, mounting, escape/freezing, boxing [reciprocal rearing with bilateral pushing]).

Statistical analysis of behavioral data

Data were analyzed using IBM SPSS (v28.0, Armonk, USA) and GraphPad Prism (v10.2.2, San Diego, USA). Longitudinal continuous data were assessed via three-way repeated-measures ANOVA (genotype × sex × age), with Greenhouse-Geisser corrections applied for violations of sphericity. For individual time points, two-way ANOVAs (genotype × sex) were used. Non-parametric data were analyzed using the aligned Rank Transform (ART) for factorial designs or Kruskal-Wallis tests for univariate comparisons. Categorical longitudinal data were analyzed via Generalized Estimating Equations (GEE). Results are presented as estimated marginal means with 95% confidence intervals. Significance was set at $p < 0.05$ (two-sided) with Bonferroni corrections for multiple comparisons. Spearman's rank correlations were used to evaluate maternal care-behavior associations. Outliers (>1.5 × interquartile range) were identified; exclusions were restricted to the PPI habituation analysis (total $n = 9$). Final group sizes for all

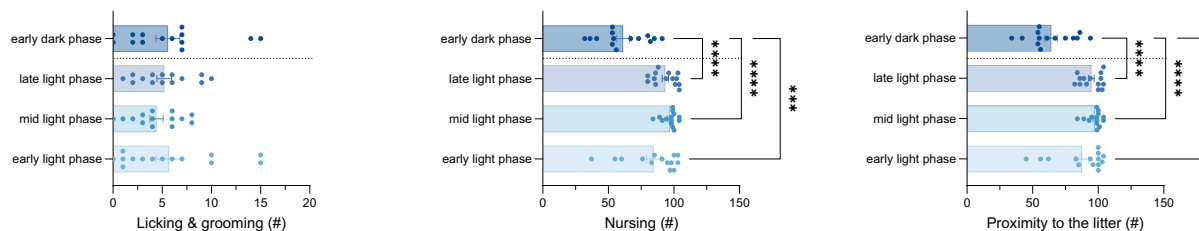
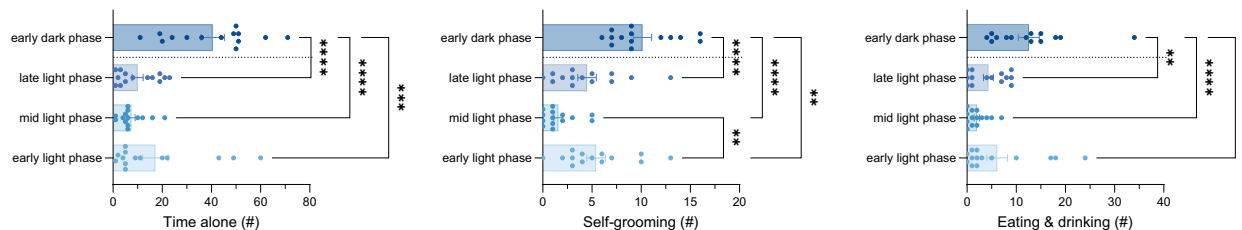
A Dam-pup interaction behavior**B** Dam-related behavior

Fig. 2 Maternal care assessment of *Tph2*^{+/-} dams during early postnatal development. Maternal care behavior was scored under a 12-h light-dark cycle (lights on at 07:00, lights off at 18:00). Observations were conducted in four daily sessions: 08:00 (early light phase), 12:00 (mid light phase), 16:00 (late light phase), and 19:30 (early dark phase) from postnatal day (P)2 to P6. Each session lasted 63 min, with observations recorded every 3 min, resulting in a total of 420 observations per dam. **A** Pup-directed behaviors: licking and grooming, nursing, and proximity to the litter. **B** Dam-focused behaviors: time spent alone, self-grooming, eating and drinking. Each dot represents one dam; a total of 14 dams were included in the analysis. Data are expressed as the mean $\pm$ standard error of the mean (SEM). Two-way ANOVA with session and behavior as factors. Effect of the session: **** $p < 0.0001$; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$. Detailed statistical comparisons are reported in Supplementary Table 2.

parameters are provided in the Supplementary Tables. All data visualizations were created in GraphPad Prism and BioRender (Toronto, Canada).

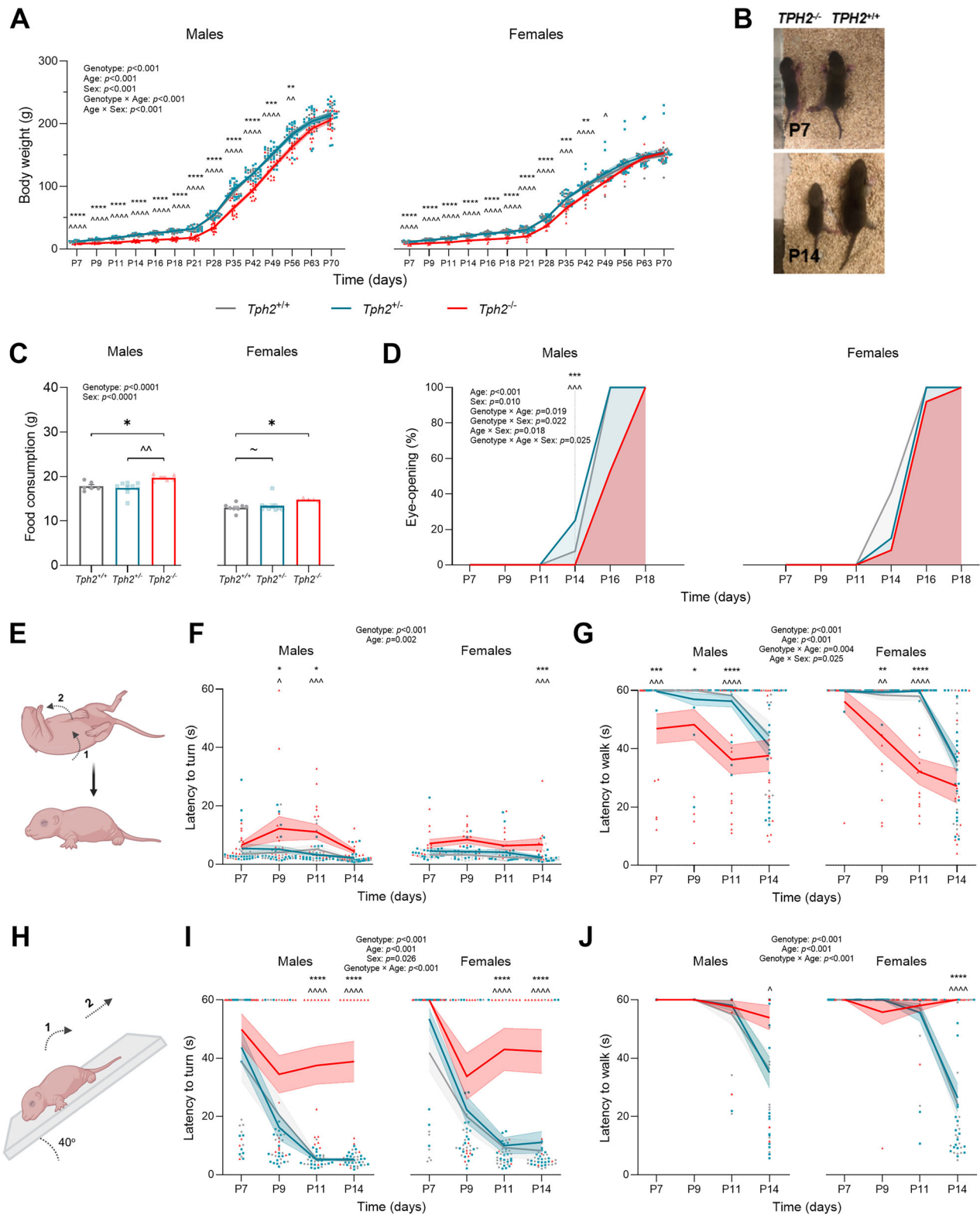
16S rRNA gene sequencing and microbiome analysis

Sample collection and DNA extraction

Following the last behavioral assessment, fecal material was collected from the distal colon (Fig. 1A), snap-frozen, and stored at -80°C . Microbiome profiling was performed in adulthood to assess established microbial community structures and to reduce variability associated with early developmental fluctuations in the microbiome. Microbial genomic DNA was extracted using standardized bead-beating and purified with a customized high-throughput protocol (BaseClear B.V., Leiden, the Netherlands). The V4 hypervariable region of the 16S rRNA gene was amplified using 515F/806R primers [48, 49] and sequenced on an Illumina NovaSeq 6000 (2×250 bp) with standard Chastity filtering.

Bioinformatics and statistical ecology

Raw sequencing reads were processed in QIIME2 [50] (v2023.7). Primers were removed with cutadapt2, and sequences were denoised, quality-filtered (Phred <30), and chimera-checked using DADA2 [51] to infer amplicon sequence variants (ASVs). Taxonomy was assigned using q2-feature-classifier [52] with a Naive Bayes classifier trained on the SILVA database (v138, 99% identity), trimmed to the V4 region [53]. Phylogenetic trees were generated using q2-phylogeny. Downstream analyses were performed in R (v4.3.0). α -diversity (ACE richness, Fisher's alpha, Gini evenness) was analyzed via multivariable linear models with genotype, sex, and their interaction as predictors. β -diversity was assessed using Euclidean distances on centered log-ratio-transformed ASV



counts, visualized by unconstrained (PCoA) and constrained (CAP) ordinations, and tested by PERMANOVA (-vegan::adonis2, 999 permutations) with Benjamin-Hochberg correction.

◀ **Fig. 3 Morphological and somatosensory reflex assessments in *Tph2*^{-/-} rats during the early postnatal period.** **A** Body weight of *Tph2*^{+/+}, *Tph2*^{+/-}, *Tph2*^{-/-} pups between postnatal day (P)7–70. **B** Representative images of *Tph2*^{+/+} and *Tph2*^{-/-} rat pups at P7 and P14. **C** Food consumption in *Tph2*^{+/+}, *Tph2*^{+/-}, *Tph2*^{-/-} rats in adulthood. **D** Eye-opening measurements of *Tph2*^{+/+}, *Tph2*^{+/-}, *Tph2*^{-/-} pups from P7 to P18. **E** Schematic of the righting reflex assay. Created with Biorender.com. **F, G** Latency to turn and to walk in the righting reflex assay for *Tph2*^{+/+}, *Tph2*^{+/-}, *Tph2*^{-/-} pups between P7–14. **H** Schematic for negative geotaxis reflex assay. Created with Biorender.com. **I, J** Upright latency to turn and to walk in the negative geotaxis assay for *Tph2*^{+/+}, *Tph2*^{+/-}, *Tph2*^{-/-} pups from P7 to P14. Sample sizes: *n* = 48 males (13 *Tph2*^{+/+}, 20 *Tph2*^{+/-}, 15 *Tph2*^{-/-}), *n* = 49 females (17 *Tph2*^{+/+}, 20 *Tph2*^{+/-}, 12 *Tph2*^{-/-}). Each dot represents one animal. Data are expressed as the mean ± standard error of the mean (SEM). Pannels **A, F, G, I, J**: Three-way repeated measures ANOVA with genotype, age, and sex as factors. Panel **C**: Generalized Estimating Equations (GEE) with a binomial distribution and logit link function; post hoc comparisons were based on estimated marginal means. Panel **D**: Kruskal-Wallis test with genotype and sex as factors. Statistical comparisons: ~ comparison of *Tph2*^{+/+} to *Tph2*^{+/-}; * comparison of *Tph2*^{+/+} to *Tph2*^{-/-}; ^ comparison of *Tph2*^{+/-} to *Tph2*^{-/-}. Detailed Bonferroni post hoc comparisons are reported in Supplementary Tables 3–9. *Tph2*, Tryptophan hydroxylase 2.

Differential abundance and prevalence modeling

Genus-level associations were evaluated using MaAsLin3 [54] with a 0.10% mean relative abundance filter. Following total sum scaling (TSS) normalization and log transformation, multivariable linear models were fitted to assess abundance and prevalence as outcomes, with genotype, sex, and their interaction as predictors. *P*-values were adjusted using the Benjamin-Hochberg procedure (FDR < 0.05). Analyses scripts are provided in Supplementary File 1.

Results

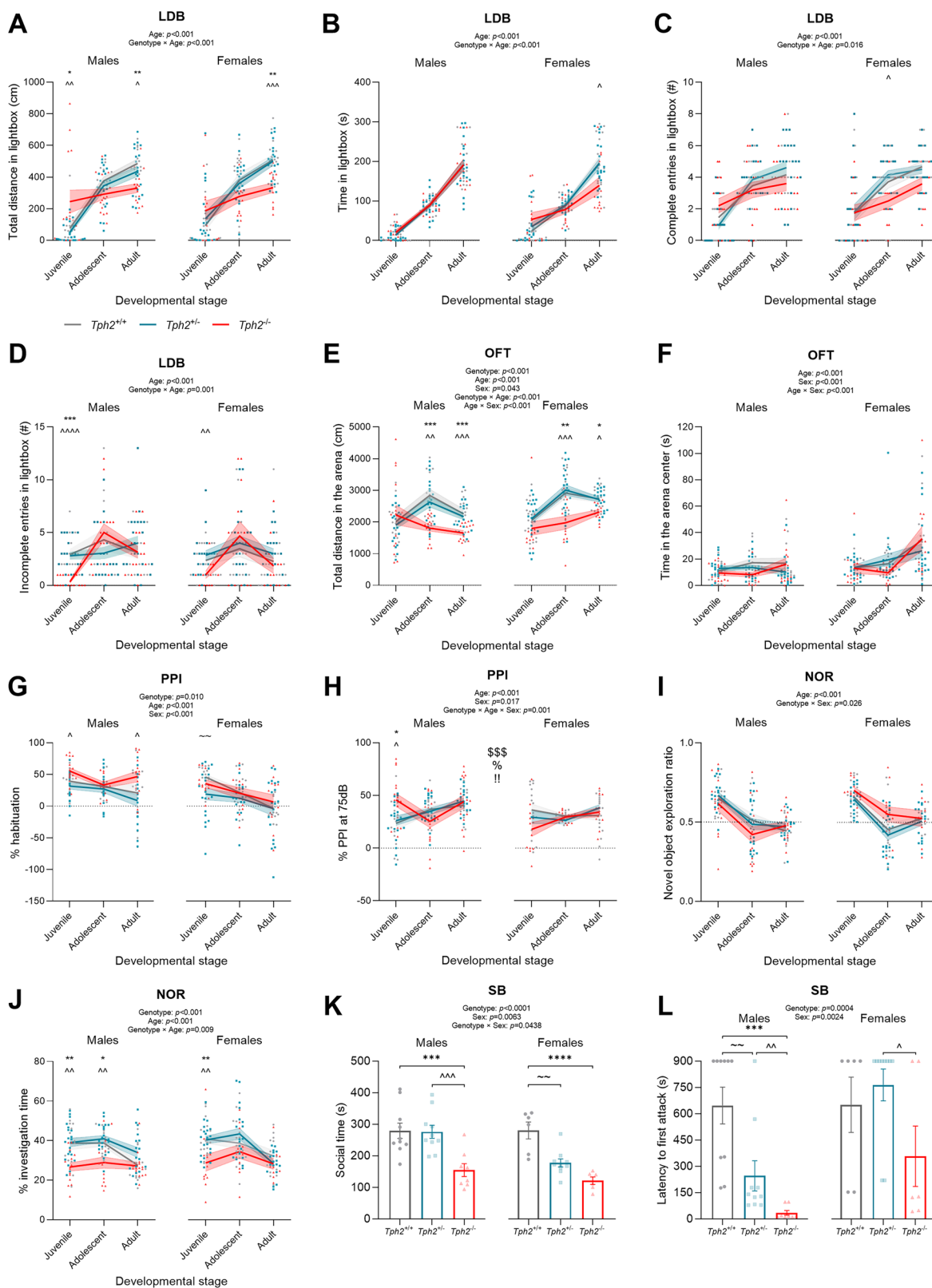
Tph2^{+/-} maternal care shows high inter-individual variance and behavior-specific circadian modulation

To evaluate the early postnatal environment of *Tph2*^{-/-} offspring, maternal care was quantified in primiparous *Tph2*^{+/+} dams from P2–P6 (Figs. 1A, 2; Supplementary Fig. 1). While nursing and proximity were uniform across dams, licking and grooming (LG) frequencies varied substantially, defining distinct care phenotypes (Supplementary Fig. 1A). Nursing and litter proximity showed circadian modulation, decreasing during the dark phase (*p* < 0.001), whereas LG remained stable across the light-dark cycle (Fig. 2A). Non-pup-directed behaviors were similarly modulated by circadian phase and showed marked inter-individual variance (Fig. 2B; Supplementary Fig. 1B) but remained stable across the 5-day assessment (data not shown). Maternal behavior did not correlate with the proportion of *Tph2*^{-/-} pups per litter (Supplementary Table 31), suggesting that these stable maternal profiles are independent of litter genotype composition or pup-directed stimuli. These stable profiles provide a basis for investigating maternal influences on offspring neurodevelopment.

Growth delay and sensorimotor reflex maturation deficits in *Tph2*^{-/-} pups

Tph2^{-/-} pups exhibited severe growth retardation from postnatal day (P)7–18 (genotype × age × sex interaction: $F_{(10,455)} = 3.255$, *p* < 0.001; Fig. 3A–B), with both sexes weighting less than *Tph2*^{+/+} and *Tph2*^{+/-} rats (*p* < 0.0001). While *Tph2*^{-/-} rats reached similar weight compared to *Tph2*^{+/+} and *Tph2*^{+/-} rats by P70, significant hyperphagia emerged ($F_{(2,39)} = 21.643$, *p* < 0.0001; Fig. 3C), with *Tph2*^{-/-} males consuming more food than *Tph2*^{+/+} (*p* = 0.0261) and *Tph2*^{+/-} (*p* = 0.0027) males and *Tph2*^{-/-} females more than *Tph2*^{+/+} (*p* = 0.0190) and *Tph2*^{+/-} female rats (*p* = 0.0189).

Developmental milestones were delayed, including later eye-opening (genotype × age × sex interaction: $\chi^2(3) = 9.334$, *p* = 0.025; Fig. 3D) and impaired neonatal reflexes (Fig. 3E–J; Supplementary Fig. 2C–H). Righting reflex latency was affected by genotype ($F_{(2,91)} = 28.124$, *p* < 0.001) and age ($F_{(3,273)} = 5.151$, *p* = 0.002; Fig. 3F), with *Tph2*^{-/-} males showing delayed righting at P9–P11 compared to both *Tph2*^{+/+} and *Tph2*^{+/-} littermates



($p < 0.05$), and females at P14 ($p < 0.001$). Conversely, $Tph2^{-/-}$ pups transitioned to walking faster post-righting (genotype $\times$ age: $F_{(6, 273)} = 3.263$, $p = 0.004$; Fig. 3G), with shorter latencies than $Tph2^{+/+}$ and $Tph2^{+/-}$ rats from P7-

◀ **Fig. 4 Altered developmental trajectories of transdiagnostic-like behaviors in *Tph2*^{-/-} rats.** **A-D** Total distance traveled, time spent, number of complete entries, and number of incomplete entries in the light compartment of the light-dark box (LDB) test for *Tph2*^{+/+}, *Tph2*^{+/-}, *Tph2*^{-/-} rats from juvenility to adulthood. **E, F** Total distance traveled in the arena and time spent in the center zone of the open field test (OFT) for *Tph2*^{+/+}, *Tph2*^{+/-}, *Tph2*^{-/-} rats from juvenility to adulthood. **G, H** Percentage of startle reflex habituation and prepulse inhibition (PPI) response to a 75 dB prepulse for *Tph2*^{+/+}, *Tph2*^{+/-}, *Tph2*^{-/-} rats from juvenility to adulthood. **I, J** Exploration ratio for the novel object and time spent investigating in the testing phase (in %) of the novel object recognition (NOR) task for *Tph2*^{+/+}, *Tph2*^{+/-}, *Tph2*^{-/-} rats from juvenility to adulthood. **K, L** Time spent engaging in social behavior (SB) and latency to first attack for *Tph2*^{+/+}, *Tph2*^{+/-}, *Tph2*^{-/-} rats in adulthood. Sample sizes: *n* = 48 males (13 *Tph2*^{+/+}, 20 *Tph2*^{+/-}, 15 *Tph2*^{-/-}), *n* = 49 females (17 *Tph2*^{+/+}, 20 *Tph2*^{+/-}, 12 *Tph2*^{-/-}). Each dot represents one animal. Data are expressed as the mean ± standard error of the mean (SEM). Panels **A-J**: Three-way repeated measures ANOVA with genotype, age, and sex as factors. Panels **K, L**: Kruskal-Wallis test with genotype and sex as factors. Statistical comparisons: ~ comparison of *Tph2*^{+/+} to *Tph2*^{+/-}; * comparison of *Tph2*^{+/+} to *Tph2*^{-/-}; ^ comparison of *Tph2*^{+/-} to *Tph2*^{-/-}. Detailed Bonferroni post hoc comparisons are reported in Supplementary Tables 14, 16–18, 22–25, 28–30. *Tph2*, Tryptophan hydroxylase 2.

11 ($p < 0.05$). At P7, *Tph2*^{-/-} males exhibited shorter latencies than *Tph2*^{-/-} females (age × sex interaction: $F_{(3, 273)} = 3.166$, $p = 0.025$). Vestibular reorientation showed a significant genotype × age interaction ($F_{(6, 273)} = 4.415$, $p < 0.001$; Fig. 3I), with *Tph2*^{-/-} pups exhibiting longer turning (P11–P14, $p < 0.0001$) and walking latencies (P14, $p < 0.05$; Fig. 3J) than *Tph2*^{+/+} and *Tph2*^{+/-} littermates. Further measures, including swimming coordination and maternal olfactory discrimination, remained largely unaffected (Supplementary Fig. 2C–H). Collectively, these findings demonstrate that TPH2 deficiency induces severe growth retardation and delayed or dysregulated maturation of somatosensory reflexes.

Age- and sex- related changes in emotional and exploratory behavior

Anxiety-related behavior was assessed using the light-dark box (LDB) at the juvenile, adolescent, and adult stages (Figs. 1A, 4A–D; Supplementary Fig. 3A). Total distance in the light compartment showed a genotype × age interaction ($F_{(4, 178)} = 11.549$, $p < 0.001$; Fig. 4A). In juvenility, *Tph2*^{-/-} males traveled farther in the light box than both *Tph2*^{+/+} ($p = 0.0378$) and *Tph2*^{+/-} male rats ($p = 0.0049$), but in adulthood, both *Tph2*^{-/-} males and females traveled shorter distances compared to *Tph2*^{+/+} and *Tph2*^{+/-} rats ($p < 0.05$). Velocity followed a similar age effect ($F_{(2, 178)} = 48.584$, $p < 0.001$; Supplementary Fig. 3A). Time spent in the light compartment showed a genotype × age interaction ($F_{(4, 178)} = 9.148$, $p < 0.001$; Fig. 4B), with *Tph2*^{-/-} females spending less time in the light box than *Tph2*^{+/-} rats in adulthood ($p = 0.0469$). Complete entries showed a genotype × age interaction ($F_{(3, 918, 174.365)} = 3.167$, $p = 0.016$; Fig. 4C), with *Tph2*^{-/-} females exhibiting less entries than *Tph2*^{+/-} rats in adolescence ($p = 0.0152$). Incomplete entries were significantly affected by a genotype × age interaction ($F_{(3, 837, 170.755)} = 4.718$, $p = 0.001$; Fig. 4D), with both male and female *Tph2*^{-/-} rats showing fewer incomplete entries in juvenility than *Tph2*^{+/+} ($p = 0.0001$) and *Tph2*^{+/-} rats ($p < 0.0001$). These findings point to a subtle developmental shift in emotional reactivity, from reduced avoidance in juvenility to diminished light-zone exploration in adulthood.

General locomotor activity and exploration were evaluated in the open field test (OFT; Figs. 1A, 4E–F; Supplementary Fig. 3B–D). Total distance traveled showed a significant genotype × age interaction ($F_{(4, 174)} = 9.987$, $p < 0.001$; Fig. 4E) and an age × sex interaction ($F_{(2, 174)} = 11.195$, $p < 0.001$). At the adolescent and adult stages, both male and female *Tph2*^{-/-} rats traveled shorter distances compared to *Tph2*^{+/+} and *Tph2*^{+/-} females ($p < 0.01$). Velocity showed a similar pattern (Supplementary Fig. 3B). Center exploration showed significant interactions for genotype × age ($F_{(3, 897, 169.520)} = 4.797$, $p = 0.001$) and age × sex ($F_{(1, 949, 169.520)} = 7.511$, $p < 0.001$; Supplementary Fig. 3C). At the adolescent stage, *Tph2*^{-/-} rats from both sexes covered shorter center distances than *Tph2*^{+/+} rats ($p < 0.05$), with *Tph2*^{-/-} females showing a smaller proportion of total distance in the center than *Tph2*^{+/+} counterparts at P35 ($p = 0.0349$; Supplementary Fig. 3D). Time spent in the center showed a significant age × sex interaction ($F_{(2, 174)} = 7.911$, $p < 0.001$), with females from all genotypes spending more time in the center in adulthood ($p < 0.05$; Fig. 4F). Together, constitutive brain 5-HT depletion modestly influences exploratory activity, marked by sex-specific differences emerging across development.

Altered and sex-specific sensorimotor gating across development

Sensorimotor gating was evaluated via prepulse inhibition (PPI) of the acoustic startle reflex at the juvenile, adolescent, and adult stages (Figs. 1A, 4G-H; Supplementary Fig. 3E-F). Acoustic startle habituation was influenced by genotype ($F_{(2, 75)} = 4.890$, $p = 0.01$), age ($F_{(2, 150)} = 13.046$, $p < 0.001$), and sex ($F_{(1, 75)} = 13.505$, $p < 0.001$; Fig. 4G). In juvenility and adulthood, *Tph2*^{-/-} males exhibited greater habituation than *Tph2*^{+/-} males and *Tph2*^{-/-} females ($p < 0.05$). At 75 dB, PPI showed a genotype $\times$ age $\times$ sex interaction ($F_{(4, 168)} = 4.810$, $p = 0.001$; Fig. 4H), with *Tph2*^{-/-} males showing higher PPI compared to *Tph2*^{+/+} males ($p = 0.0393$), *Tph2*^{+/-} males ($p = 0.0484$), and *Tph2*^{-/-} females ($p = 0.0025$) in juvenility. At 80 dB, PPI was affected by genotype $\times$ age ($F_{(4, 170)} = 3.301$, $p = 0.012$), genotype $\times$ sex ($F_{(2, 85)} = 3.831$, $p = 0.026$), and genotype $\times$ age $\times$ sex ($F_{(4, 170)} = 2.452$, $p = 0.048$; Supplementary Fig. 3E). *Tph2*^{+/-} males displayed greater inhibition than *Tph2*^{+/+} males in adolescence ($p = 0.0042$), whereas *Tph2*^{-/-} females showed reduced PPI compared to their male counterparts in juvenility ($p = 0.0115$). At 85 dB, a genotype $\times$ age interaction persisted ($F_{(4, 176)} = 2.976$, $p = 0.021$; Supplementary Fig. 3F), with *Tph2*^{-/-} males exhibiting higher PPI than females in juvenility ($p = 0.0346$), and *Tph2*^{+/-} males maintaining higher inhibition than females in adolescence and adulthood ($p < 0.05$). Overall, these data reveal male-specific PPI enhancement in *Tph2*^{-/-} rats during juvenility, particularly at lower prepulse intensities, suggesting that lifelong central 5-HT deficiency modestly influences sensorimotor gating in a sex-dependent manner.

Tph2-deficiency reduces object exploration across development

Recognition memory and object discrimination were assessed in the novel object recognition (NOR) task at the juvenile, adolescent, and adult stages (Figs. 1A, 4I, J). The novel object exploration ratio showed a significant genotype $\times$ sex interaction ($F_{(2, 79)} = 3.806$, $p = 0.026$; Fig. 4I). In juvenility and adolescence, *Tph2*^{-/-} males displayed lower exploration ratios than *Tph2*^{-/-} females ($p < 0.05$). A similar sex difference was observed in *Tph2*^{+/+} rats in adulthood ($p = 0.0428$). In adolescence, discrimination ratios across groups largely converged toward the 0.5 chance level, indicating a lack of novelty preference during adolescence and adulthood. Total investigation time during testing was affected by a significant genotype $\times$ age interaction ($F_{(4, 158)} = 3.535$, $p = 0.009$; Fig. 4J). In juvenility, both male and female *Tph2*^{-/-} rats spent less time exploring objects than *Tph2*^{+/+} and *Tph2*^{+/-} littermates ($p < 0.01$). This reduction persisted in adolescence, with *Tph2*^{-/-} males continuing to show lower investigation times than *Tph2*^{+/+} and *Tph2*^{+/-} male rats ($p < 0.05$). Together, these results indicate that *Tph2* deletion decreases total novel investigation time across development, whereas discrimination ratios remain independent of genotype. This pattern likely reflects reduced exploratory behavior or locomotor activity, rather than a deficit in recognition memory.

Reduced social interaction and increased aggression in *Tph2*^{-/-} rats

Social behavior was evaluated in adulthood (Figs. 1A, 4K-L; Supplementary Fig. 3G, I). Social interaction time showed a genotype $\times$ sex interaction ($F_{(2, 44)} = 3.361$, $p = 0.0438$; Fig. 4K). *Tph2*^{-/-} males spent less time engaging socially than both *Tph2*^{+/+} and *Tph2*^{+/-} males ($p = 0.0002$), a pattern mirrored in *Tph2*^{-/-} females compared to *Tph2*^{+/+} littermates ($p < 0.0001$). Within the *Tph2*^{+/-} group, males displayed higher sociability than females ($p = 0.0022$), a sex difference corroborated by the number of social contacts (Supplementary Fig. 3G). Latency to first attack followed similar effects, with effects of genotype ($F_{(2, 44)} = 9.433$, $p = 0.0004$) and sex ($F_{(1, 44)} = 10.362$, $p = 0.0024$; Fig. 4L). *Tph2*^{-/-} males initiated attacks faster than *Tph2*^{+/+} ($p < 0.0001$) and *Tph2*^{+/-} rats ($p = 0.0301$). Among females, *Tph2*^{-/-} rats also displayed shorter attack latencies than *Tph2*^{+/-} animals ($p = 0.0135$). Aggressive behavior was further modulated by a significant genotype $\times$ sex interaction ($F_{(2, 44)} = 12.835$, $p < 0.0001$; Supplementary Fig. 3H). *Tph2*^{-/-} males exhibited more aggression than *Tph2*^{+/+} ($p < 0.0001$) and *Tph2*^{+/-} rats ($p = 0.0437$), while *Tph2*^{+/-} males showed increased aggression relative to *Tph2*^{+/+} counterparts ($p = 0.0329$). Across both *Tph2*^{+/-} and *Tph2*^{-/-} groups, males displayed higher aggression times and attack frequencies than

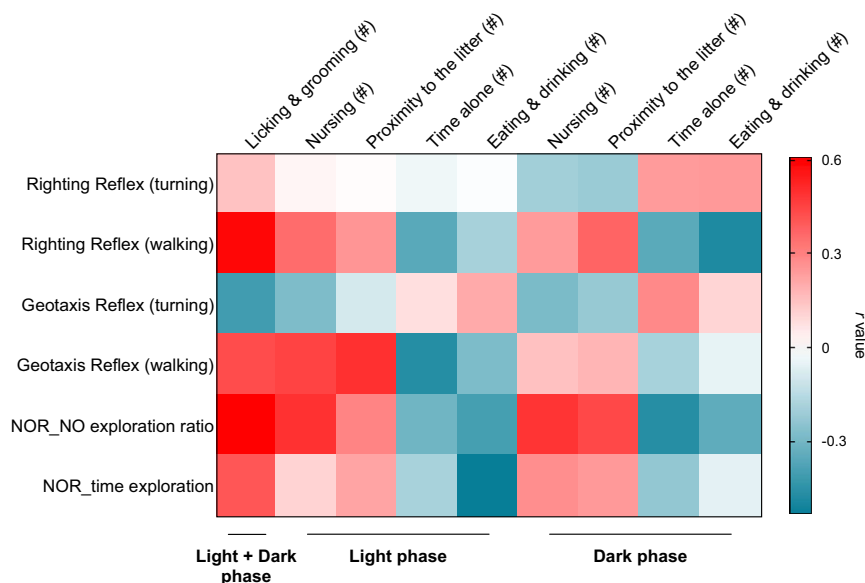


Fig. 5 Associations between maternal caregiving behaviors and offspring behavioral outcomes. Spearman's rank correlation analysis was performed to assess relationships between maternal care variables and selected statistically significant offspring behavioral outcomes. Pairwise Spearman's correlation coefficients (r) are color-coded: red indicates positive correlations; blue indicates negative correlations. Offspring behavioral change scores were calculated as the difference between the final and first testing days for each parameter. To account for litter effects, behavioral outcomes were averaged per litter ($n = 14$). The matrix illustrates the strength and direction of these relationships. Sample sizes: $n = 48$ males (13 $Tph2^{+/+}$, 20 $Tph2^{+/-}$, 15 $Tph2^{-/-}$), $n = 49$ females (17 $Tph2^{+/+}$, 20 $Tph2^{+/-}$, 12 $Tph2^{-/-}$). All Spearman's r values and corresponding p -values are reported in Supplementary Table 32. NOR, novel object recognition.

females ($p < 0.01$; Supplementary Fig. 3I). In sum, $Tph2$ deficiency leads to reduced social interaction and increased aggression, as evidenced by higher attack frequency and shorter attack latencies, particularly in males.

Maternal care behavior correlates with later offspring sensorimotor and exploratory outcomes

To investigate the developmental impact of early caregiving, Spearman's correlations were performed between maternal behaviors and offspring performance (Fig. 5). To account for litter effects, behavioral outcomes were averaged per litter ($n = 14$). Behavioral change scores were calculated as the difference between the final and initial test days. Light- and dark phase LG were pooled due to minimal variance between sessions, whereas light/dark phases remained distinct for other maternal parameters. Total LG correlated positively with offspring latency to walk in the righting reflex ($r = 0.601$, $p = 0.023$), and the novel object exploration ratio ($r = 0.616$, $p = 0.019$; Fig. 5). Although other associations did not reach statistical significance, notable trends were observed. Light-phase nursing and litter proximity were positively associated with novel object preference and negative geotaxis performance, respectively (nursing: $r = 0.496$, $p = 0.072$; proximity: $r = 0.499$, $p = 0.072$), with commensurate trends during the dark phase. Conversely, time spent alone was negatively associated with sensorimotor and exploratory outcomes (light phase: $r = -0.477$, $p = 0.087$; dark phase: $r = -0.4759$, $p = 0.086$). Maternal eating and drinking also showed negative associations with object investigation time (light phase: $r = -0.541$, $p = 0.046$) and righting reflex latency (dark phase: $r = -0.492$, $p = 0.074$). Together, these findings suggest that higher-quality maternal care, particularly LG, is associated with improved sensorimotor performance and novel object recognition memory in the offspring.

Altered gut microbial diversity and taxonomic abundance in the absence of TPH2

To identify microbial signatures associated with $Tph2$ deficiency, we characterized gut microbial diversity and composition (Figs. 1A, 5–7; Supplementary Figs. 4–6). Within-sample richness (α -diversity) indices showed high

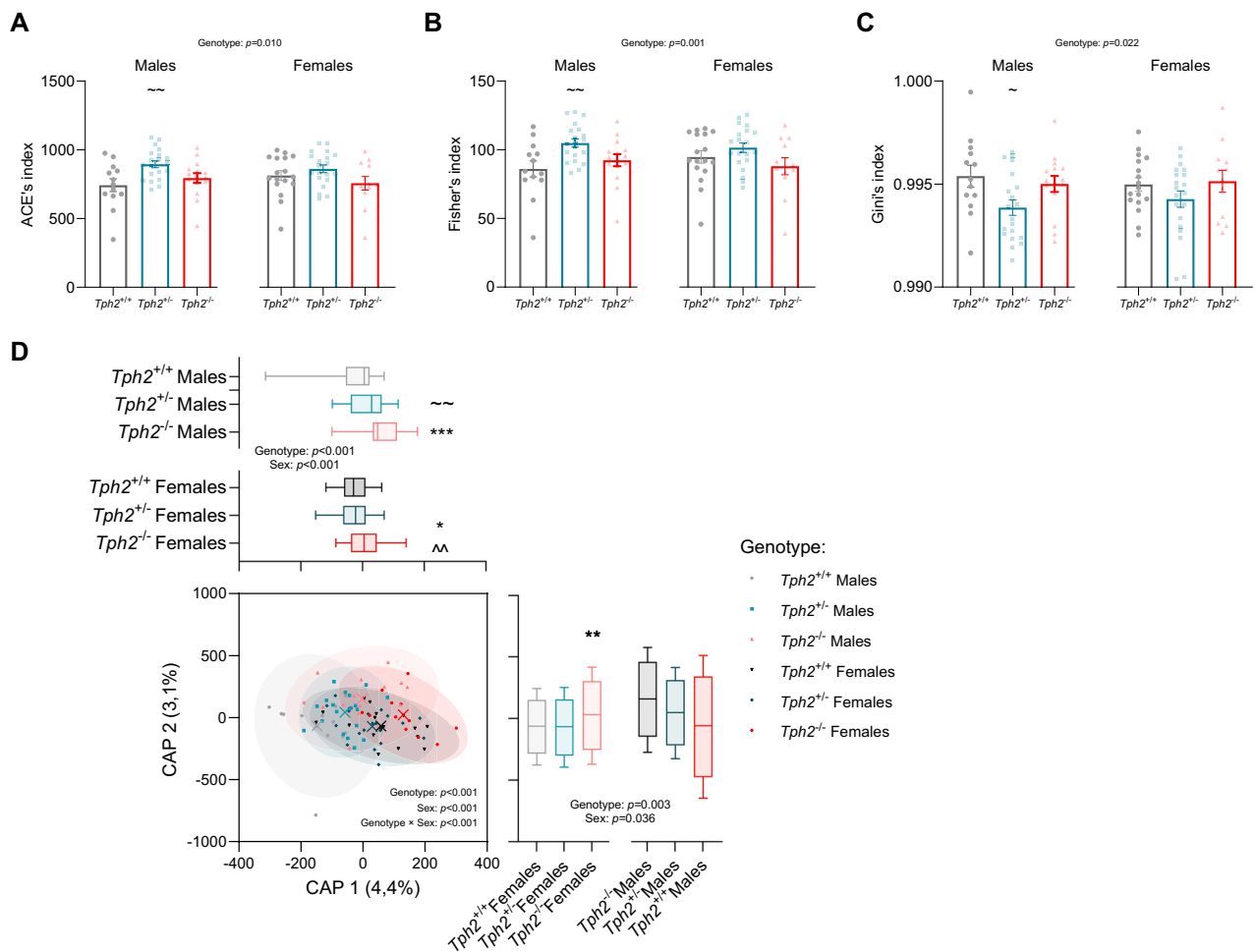
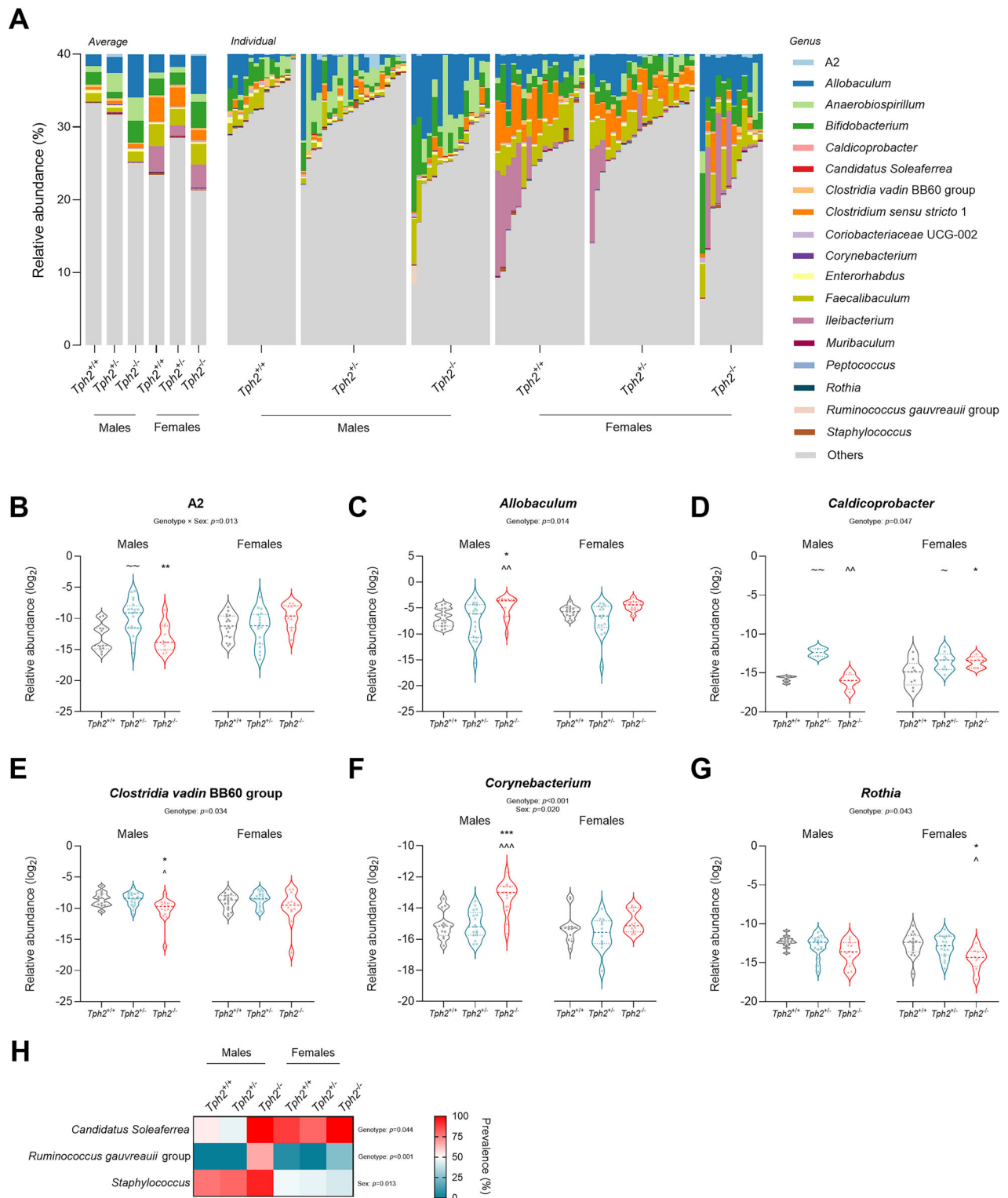


Fig. 6 Effects of TPH2 genotype on gut microbial α - and β -diversity. **A–C** α -diversity was assessed using the abundance-based coverage estimator (ACE; richness), Fisher's index (diversity), and Gini's index (dominance). Diversity metrics are expressed as the mean $\pm$ standard error of the mean (SEM). Each dot represents one animal. Two-way ANOVA with genotype and sex as factors. Statistical comparisons: ~ comparison of $Tph2^{+/+}$ to $Tph2^{+/-}$. **D** Microbial community composition was visualized with canonical analysis of principal coordinates (CAP), with ordination constrained by genotype ($Tph2^{+/+}$, $Tph2^{+/-}$, and $Tph2^{-/-}$) and sex (male, female). Ellipses represent 95% confidence intervals around group centroids. Sample sizes: $n = 48$ males (13 $Tph2^{+/+}$, 20 $Tph2^{+/-}$, 15 $Tph2^{-/-}$), $n = 49$ females (17 $Tph2^{+/+}$, 20 $Tph2^{+/-}$, 12 $Tph2^{-/-}$). Detailed Bonferroni post hoc comparisons are reported in Supplementary Tables 33–34. *Tph2*, Tryptophan hydroxylase 2.

internal correlation (Supplementary Fig. 4); accordingly, the abundance-based coverage estimator (ACE), Fisher's alpha, and Gini indices (reflecting richness and dominance) provided the most representative data. ACE ($F_{(2, 91)} = 6.13$, $p = 0.01$), Fisher's alpha ($F_{(2, 91)} = 6.32$, $p = 0.001$), and Gini ($F_{(2, 91)} = 4.74$, $p = 0.022$) differed by genotype, with no genotype $\times$ sex interactions (Fig. 6A–C). Post hoc analysis revealed that $Tph2^{+/-}$ males exhibited greater richness (ACE: $p = 0.009$; Fisher: $p = 0.010$), and reduced dominance (Gini: $p = 0.037$) relative to $Tph2^{+/+}$ males. In contrast, evenness (Camargo, Evar) and Rarity indices showed no genotype effects (Supplementary Fig. 5A–C), indicating that *Tph2*-related α -diversity shifts are largely restricted to community richness. While exploring the functional relevance of these signatures, microbial richness was positively associated with novel object recognition performance in adulthood, whereas increased microbial dominance linked to impaired cognitive outcomes (Supplementary Results).

Between-sample community structure (β -diversity), visualized via constrained analysis of principal coordinates (CAP), showed clustering by genotype and sex (7.5% total variance; Fig. 6D). Unconstrained PCoA accounted for 18.1% of the variation, with more modest group separation (Supplementary Fig. 5D). PERMANOVA confirmed significant effects of genotype ($F_{(2, 91)} = 2.22$, $p < 0.001$), sex ($F_{(1, 91)} = 2.90$, $p < 0.001$), and their interaction ($F_{(2, 91)} = 2.22$, $p < 0.001$).



$g_{11}) = 1.62$, $p < 0.001$). Sensitivity analyses across multiple model specifications yield consistent results (Supplementary File 1). Post hoc testing showed that $Tph2^{+/-}$ ($p = 0.006$) and $Tph2^{-/-}$ ($p < 0.001$) males differed from $Tph2^{+/+}$ counterparts, while among females, $Tph2^{-/-}$ rats differed from both $Tph2^{+/+}$ ($p = 0.046$) and $Tph2^{+/-}$ ($p = 0.007$) littermates.

◀ **Fig. 7 TPH2 genotype-dependent alterations in gut microbiome taxonomic composition.** **A** Relative abundances of the eighteen differently expressed bacterial genera after MasASLin3 analysis. For each group, stratified by genotype and sex, the mean relative abundance is shown on the left, followed by the individual sample values for each animal within the group. **B–G** Selected genera showing significant genotype-associated differences: A2, *Allobaculum*, *Caldicoprobacter*, *Clostridia vadin* BB60 group, *Corynebacterium*, and *Rothia*. Sample sizes: $n = 48$ males (13 $Tph2^{+/+}$, 20 $Tph2^{+/-}$, 15 $Tph2^{-/-}$), $n = 49$ females (17 $Tph2^{+/+}$, 20 $Tph2^{+/-}$, 12 $Tph2^{-/-}$). Each dot represents one animal. Data are expressed as the mean $\pm$ standard error of the mean (SEM). MasASLin3 with genotype and sex as factors. Statistical comparisons: ~ comparison of $Tph2^{+/+}$ to $Tph2^{+/-}$; * comparison of $Tph2^{+/+}$ to $Tph2^{-/-}$; ^ comparison of $Tph2^{+/-}$ to $Tph2^{-/-}$. **H** Prevalence of subdominant gut bacterial genera in $Tph2^{-/-}$ rats. Bacterial genera shown were not detected in all samples across groups. Prevalence is expressed as the percentage of animals in which each genus was present. Detailed Bonferroni post hoc comparisons are reported in Supplementary Table 36. *Tph2*, Tryptophan hydroxylase 2.

Taxonomic profiling identified eighteen differentially abundant taxa out of 156 genera (Fig. 7A). Genus A2 showed a significant genotype $\times$ sex interaction ($p = 0.013$; Fig. 7B). Relative to $Tph2^{+/+}$ males, $Tph2^{-/-}$ littermates were enriched in *Allobaculum* ($p = 0.014$) and *Corynebacterium* ($p < 0.001$), whereas *Caldicoprobacter* ($p = 0.047$), *Clostridia vadin* BB60 group ($p = 0.034$) and *Rothia* ($p = 0.043$) were depleted in $Tph2^{-/-}$ males and females, respectively (Fig. 7C–G). Ten genera differed by sex, including enriched levels of *Anaerobiospirillum* ($p = 0.043$), *Enterorhabdus* ($p = 0.002$), and *Coriobacteriaceae* UCG-002 ($p = 0.027$) in males (Supplementary Fig. 6).

Prevalence modeling further identified *Candidatus Soleaferrea* ($p = 0.044$) and the *Ruminococcus gauvreauii* group ($p < 0.001$) as genotype-dependent, both increased in $Tph2^{-/-}$ rats, while *Staphylococcus* differed by sex ($p = 0.013$; Fig. 7H). Collectively, these findings indicate that *Tph2* deficiency influences gut microbial ecology by modulating richness, community structure, and genus-level composition in a sex-dependent manner.

Discussion

This study provides a longitudinal characterization of the developmental, behavioral, and ecological consequences of lifelong central 5-HT deficiency ($Tph2^{-/-}$) in rats. Loss of brain 5-HT disrupted early somatic growth and sensorimotor maturation, followed by developmental alterations in emotional regulation, social behavior, and gut microbial composition. Notably, the observed shifts in microbial richness and community structure suggest that central 5-HT deficiency extends beyond primary neural circuits to influence the systemic environment during critical periods of neurodevelopment. Together, these findings highlight the multisystem role of 5-HT in shaping neurodevelopmental behavioral trajectories across genotype, sex, and early-life environmental contexts.

Consistent with previous work, early postnatal growth retardation in $Tph2^{-/-}$ pups emerged as a robust phenotype, accompanied by delayed maturation of sensorimotor reflexes [16, 17, 22, 23, 26, 27, 55, 56]. Our data extends these findings by showing that these delays include qualitative alterations in motor performance rather than a uniform developmental lag. Prolonged righting, impaired negative geotaxis, and postural instability point to deficits in the coordination and regulation of motor output. Remarkably, shorter latencies to initiate walking post-righting in $Tph2^{-/-}$ pups suggest dysregulated motor control, potentially reflecting altered maturation of excitatory-inhibitory balance within developing motor circuits, a process influenced by early 5-HT signaling [3, 4]. Subtle sex-specific differences in swimming posture at later time points, together with reduced performance in the bar-holding assay in this model [17], further support persistent alterations in muscular tone and neuromuscular coordination. Collectively, these converging sensorimotor findings suggest that early 5-HT deficiency disrupts the maturation and integration of motor control systems during postnatal development, rather than simply delaying milestone acquisition. In contrast to previous reports of altered maternal odor discrimination in *Tph2*-deficient models [17, 55, 56], no genotype-driven differences were detected in early olfactory preference. This discrepancy likely reflects methodological differences, as simpler choice-based paradigms may lack sensitivity to detect fine-grained spatial or temporal exploration deficits.

Across later developmental stages, TPH2 deficiency was associated with alterations in emotional regulation and exploratory behavior. The observed shift from reduced avoidance of the light compartment during juvenility to decreased exploration in adulthood suggests that 5-HT loss affects exploratory behavior and general activity levels in

a developmentally contingent manner. Prior studies have reported mixed anxiety-related phenotypes in *Tph2*^{-/-} rodents, ranging from reduced anxiety-like behavior [25, 29] to no detectable differences [28]. Strain-dependent differences in anxiety-like and exploratory behaviors have also been reported in rats [57], which may contribute to variability in behavioral outcomes and limit the direct generalization of results across genetic backgrounds [58]. Such variability likely reflects differences in developmental timing, task demands, and/or the specific neural circuits engaged by each assay. Additionally, circadian- or time-of-day modulation of exploratory behavior and locomotor activity may influence these outcomes. Our findings support the view that lifelong 5-HT dysregulation does not produce a stable anxiety-like phenotype but rather alters the maturation and expression of emotional regulation across development.

Sensorimotor gating and cognition were modestly affected. Enhanced PPI at lower prepulse intensities in *Tph2*^{-/-} males during early development suggests subtle, sex-specific alterations in inhibitory processing, consistent with emerging evidence implicating 5-HT signaling in the maturation of sensorimotor gating circuits [59]. In contrast, preserved novel object recognition performance, combined with reduced total exploration time in *Tph2*^{-/-} rats, suggests changes in exploratory behavior or decreased locomotor activity rather than a primary recognition memory impairment, consistent with previous findings [55].

In adulthood, particularly in males, *Tph2*^{-/-} rats displayed reduced social interaction, heightened aggression, and faster attack initiation. These results replicate and extend previous reports of sex-dependent alterations in social behavior in 5-HT models [28, 29, 60], reinforcing the role of 5-HT in modulating social motivation and inhibitory control. Social interaction was assessed exclusively in adulthood; consequently, juvenile social play behavior, which peaks during early developmental stages in rats, was not evaluated. Although depressive-like behaviors were also not directly assessed, incorporating reward- and stress-related assays in future work will be crucial to further characterize affective phenotypes associated with lifelong central 5-HT deficiency.

Beyond genotype-driven traits, natural variation in early maternal care emerged as a predictor of offspring sensorimotor and exploratory outcomes. LG and nursing are well-established regulators of early neurodevelopment [61–63] and were positively associated with behavioral performance. While caregiving was not experimentally manipulated here, and thus we cannot determine whether it directly modulates specific *Tph2*^{-/-} behavioral phenotypes, the overlap between this sensitive postnatal window and key neurodevelopmental processes, including synaptogenesis and shifts in 5-HT signaling [4], suggests heightened susceptibility to environmental modulation. These findings highlight maternal care as an important environmental factor that may interact with 5-HT tone to shape developmental trajectories. This is supported by evidence that reduced ultrasonic calling in *Tph2*^{-/-} pups can lead to reduced maternal caregiving, whereas environmental enrichments, such as communal nesting, can improve behavioral outcomes [17, 18]. Future studies employing cross-fostering, environmental enrichment, or related rearing paradigms will be essential to directly disentangle the contributions of genetic and early environmental influences and to test genotype-dependent sensitivity to caregiving quality.

At the ecological level, we identified genotype- and sex-dependent alterations in gut microbiota composition, suggesting that central 5-HT tone exerts downstream influences on host-microbiome interactions. Notably, *Tph2*^{+/-} males exhibited the most pronounced changes in microbial diversity, potentially reflecting increased sensitivity of gut-brain signaling under partial 5-HT loss. This observation aligns with previous studies linking TPH2-mediated 5-HT signaling to metabolic regulation and microbiota composition [64, 65]. Although TPH2 is predominantly expressed in central 5-HT neurons, its presence in enteric neurons of the myenteric plexus, together with reciprocal regulation between microbial metabolites and peripheral 5-HT pools, supports bidirectional mechanisms linking 5-HT signaling to gut homeostasis [66, 67].

Taxonomic differences further refine this interpretation. Enrichment of *Allobaculum* in *Tph2*^{-/-} males, previously associated with lean metabolic profiles [68] and butyrate production [69–71], may represent a compensatory adaptation to central 5-HT deficiency via short-chain fatty acid (SCFA)-mediated modulation of peripheral 5-HT signaling and brain plasticity [34, 72]. In contrast, depletion of the *Clostridia* vadin BB60 group, previously linked to stress-related dysbiosis [73], together with enrichment of *Corynebacterium* in *Tph2*^{-/-} males, associated with neuroinflammation and depressive-like behaviors [74], suggest that central 5-HT deficiency may bias microbial communities toward stress-associated profiles. Widespread sex-specific effects across taxa reinforce the idea that sex hormones and

5-HT signaling jointly shape microbial organization, consistent with known sexual dimorphism in 5-HT systems and psychiatric vulnerability [75–77].

By integrating behavioral, caregiving, and microbiota data across development, this study demonstrates how lifelong central 5-HT deficiency interacts with sex and early postnatal environmental factors to shape neurodevelopmental trajectories. These findings are relevant to human neuropsychiatric conditions in which 5-HT dysfunction and early-life adversity increase disease risk, particularly those characterized by changes in emotional regulation, social functioning, and stress responsivity throughout development [78, 79]. Our study underscores the role of 5-HT signaling in early sensorimotor and social-emotional development and highlights the gut microbiota as a potential contributor to alterations in feeding behavior and metabolic regulation in adulthood. Although behavioral testing was standardized to the light phase, the influence of circadian modulation on exploration and locomotor output cannot be entirely excluded. Future studies assessing behavior across circadian phases will be important to refine these interpretations. Subsequent research should integrate experimental manipulation of early caregiving, comprehensive profiling of maternal and offspring microbiota, and longitudinal metabolomic analyses to clarify causal mechanisms linking central 5-HT signaling to developmental trajectories of vulnerability and resilience. Cross-fostering designs and maternal microbiota profiling will be essential to distinguish genetic, environmental, and microbial influences on early developmental programming.

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41398-026-04354-2>.

Acknowledgements The authors thank the students, specifically Thibault A. Merkelijn and Aiswarya R. Nair, and technical staff at the Centraal Dierenlaboratorium (CDL; Radboud University Medical Center, Nijmegen, The Netherlands) for their valuable scientific input and technical assistance.

Author contributions MCFS acquired the data, performed statistical analysis, designed and executed figures and tables, wrote the original draft, and edited and reviewed the manuscript. RCRC supported data acquisition, statistical analysis, manuscript writing, and editing. TL and LB helped with data acquisition and statistical analysis. RCRC, EEN, and AAV supported microbiome data analysis. JRH and SMK designed the study, acquired funding, supervised the work, and supported manuscript writing. RCRC, TL, EEN, LB, NA, MB, TW, MW, AAV, JRH, and SMK read, critically revised, and approved the final manuscript.

Funding This work is part of the Marie Skłodowska-Curie Action Innovative Training Network *Serotonin and Beyond* under the European Union's Horizon 2020 research and innovation program (Grant Agreement 953327). It was further supported by grants from the Dutch Research Council (NWO) for the ProMiSe NWA project (Project ID: NWA.1160.18.320) and by the Donders Institute for Brain, Cognition and Behavior (Radboud University Nijmegen, The Netherlands), through the Donders Research Stimulation Fund, both awarded to SMK.

Data availability The datasets generated and analyzed for the current study will be made available in the Radboud Repository upon publication of this manuscript.

Competing interests The authors declare that they have no conflict of interest.

Ethics approval All procedures and animal-handling methods were conducted in accordance with relevant institutional and national guidelines and regulations (EU Directive 2010/63/EU and the Dutch Animal Testing Acts). All experimental protocols were approved by the Central Authority for Scientific Procedures on Animals (CCD) under project license AVD10300 2020 1142.

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