

Supplementary Information

Effects of constitutive depletion or excess of serotonin during development on the maturation of prefrontal monoaminergic circuits

Original Research Article

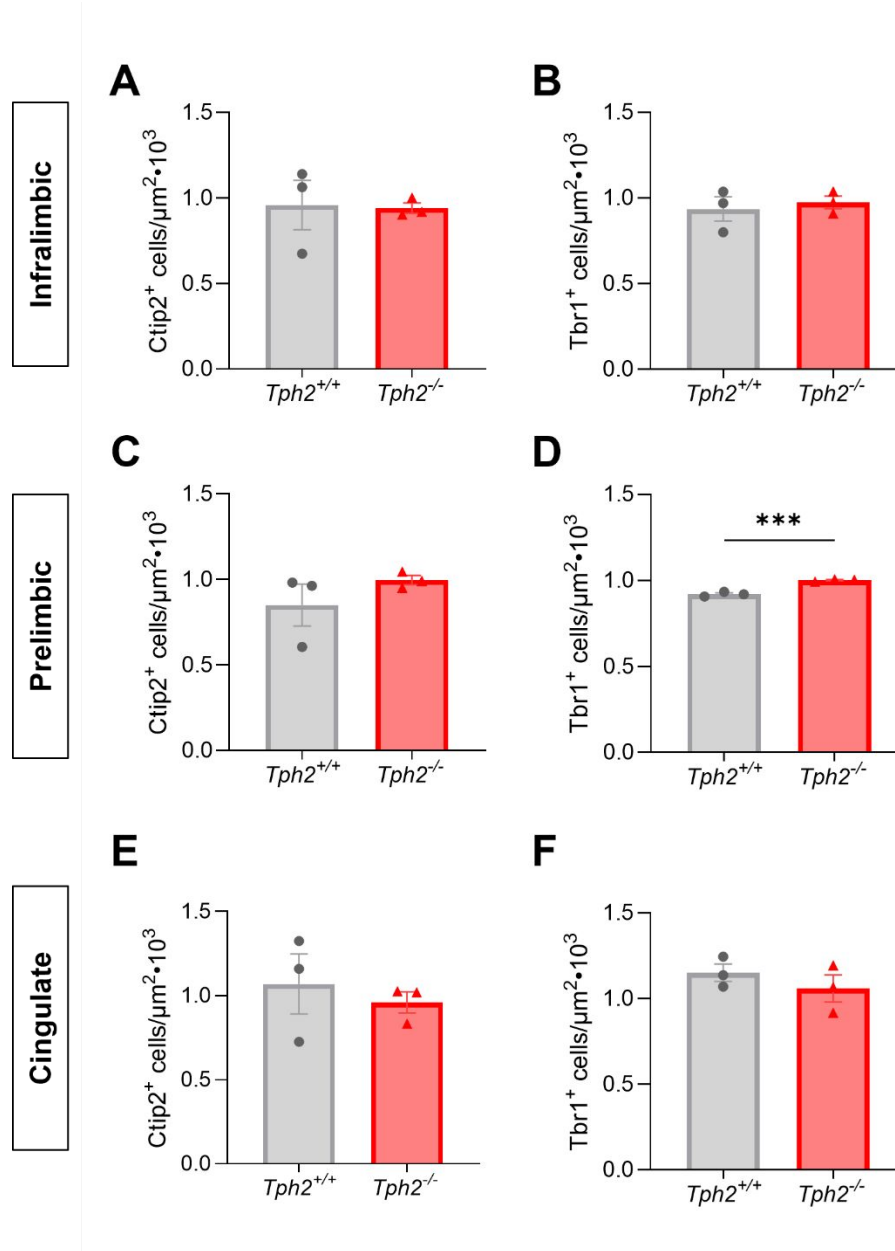
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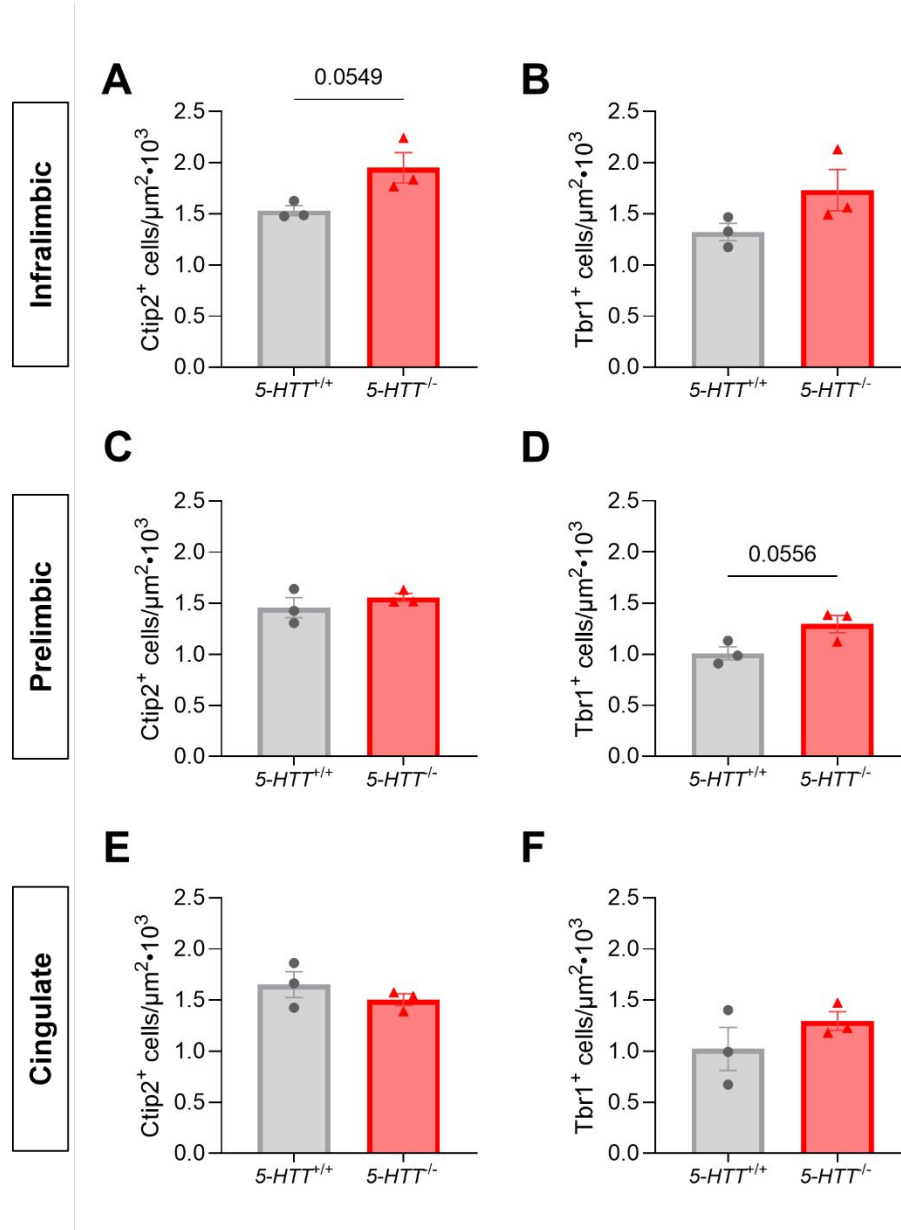
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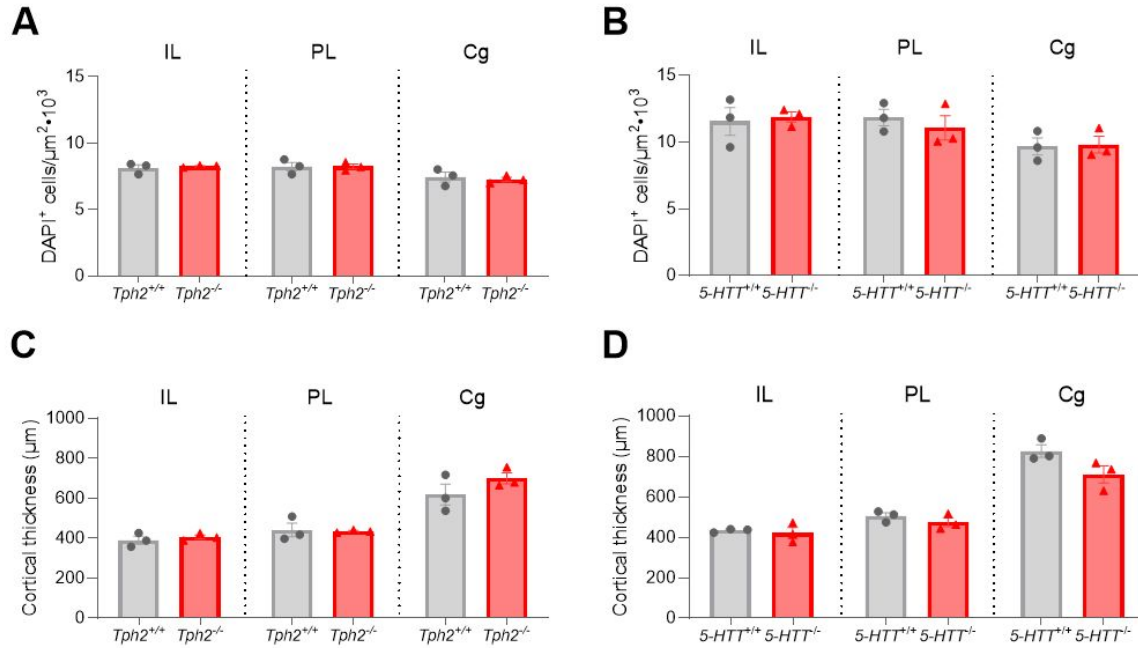
Supplementary Figures



Supplementary Figure 1. Prefrontal cytoarchitecture is selectively altered in the prelimbic cortex in the absence of *Tph2*. (A-F) Quantification of total Ctip2⁺ and Tbr1⁺ cells, presented as raw counts normalized to the total cortical area within the infralimbic (IL), prelimbic (PL), and cingulate (Cg) cortices of *Tph2*^{-/-} compared to *Tph2*^{+/+} embryos. Each data point represents one animal. Data are presented as the mean ± standard error of the mean (SEM). Statistical analysis was performed using the unpaired two-tailed Student's *t*-test. Statistical significance: **p* < 0.05 for *Tph2*^{+/+} compared to *Tph2*^{-/-}. *Tph2*: Tryptophan hydroxylase 2.

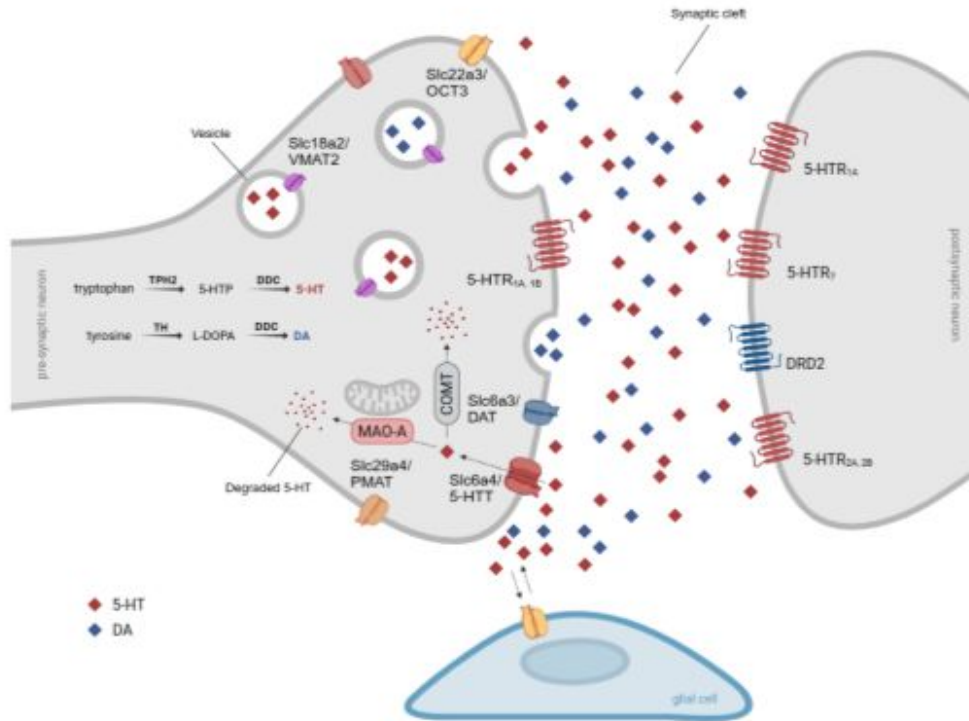


Supplementary Figure 2. Deep-layer neuronal density is largely preserved in 5-HTT^{-/-} embryos. (A-F) Quantification of total Ctip2⁺ and Tbr1⁺ cells, presented as raw counts normalized to the total cortical area within the infralimbic (IL), prelimbic (PL), and cingulate (Cg) cortices of 5-HTT^{-/-} compared to 5-HTT^{+/+} embryos. Each data point represents one animal. Data are presented as the mean $\pm$ standard error of the mean (SEM). Statistical analysis was performed using the unpaired two-tailed Student's *t*-test. 5-HTT: serotonin transporter.

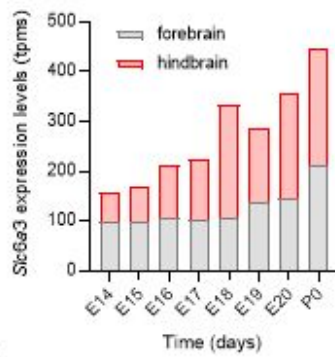


Supplementary Figure 3. Total DAPI⁺ cell density and cortical thickness are preserved across models of 5-HT imbalance. (A-B) Quantification of total DAPI⁺ cell density across bins in the infralimbic (IL), prelimbic (PL), and cingulate (Cg) cortices of (A) *Tph2*^{-/-} compared to *Tph2*^{+/+} embryos and (B) of *5-HTT*^{-/-} compared to *5-HTT*^{+/+} rats. (C-D) Evaluation of total cortical thickness (measured from the ventricular surface to the pial surface) in the IL, PL, and Cg cortices of (C) *Tph2*^{-/-} and (D) *5-HTT*^{-/-} models. Sample size: $n = 12$ males (3 *Tph2*^{+/+}, 3 *Tph2*^{-/-}, 3 *5-HTT*^{+/+}, 3 *5-HTT*^{-/-}). Each data point represents one animal. Data are presented as the mean $\pm$ standard error of the mean (SEM). Statistical analyses were performed using two-way ANOVA with medial prefrontal cortex (mPFC)-subregion and genotype as factors. *Tph2*: tryptophan hydroxylase 2, *5-HTT*: serotonin transporter.

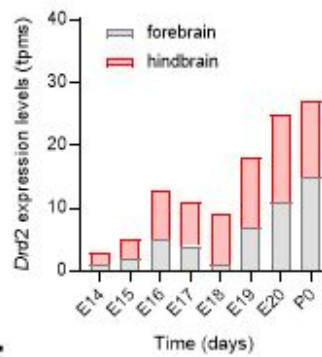
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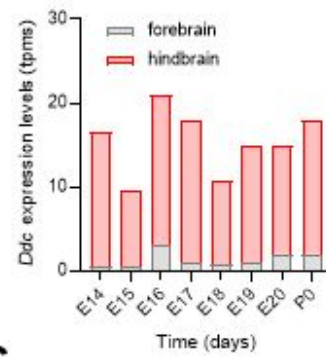
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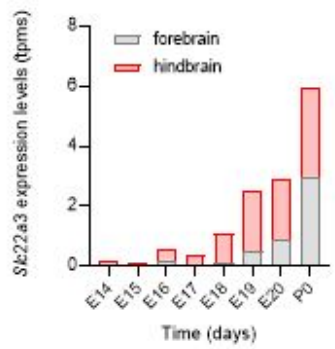
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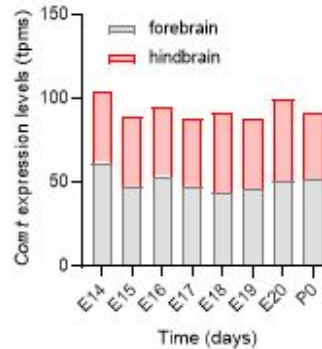
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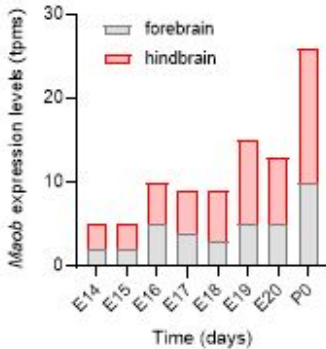
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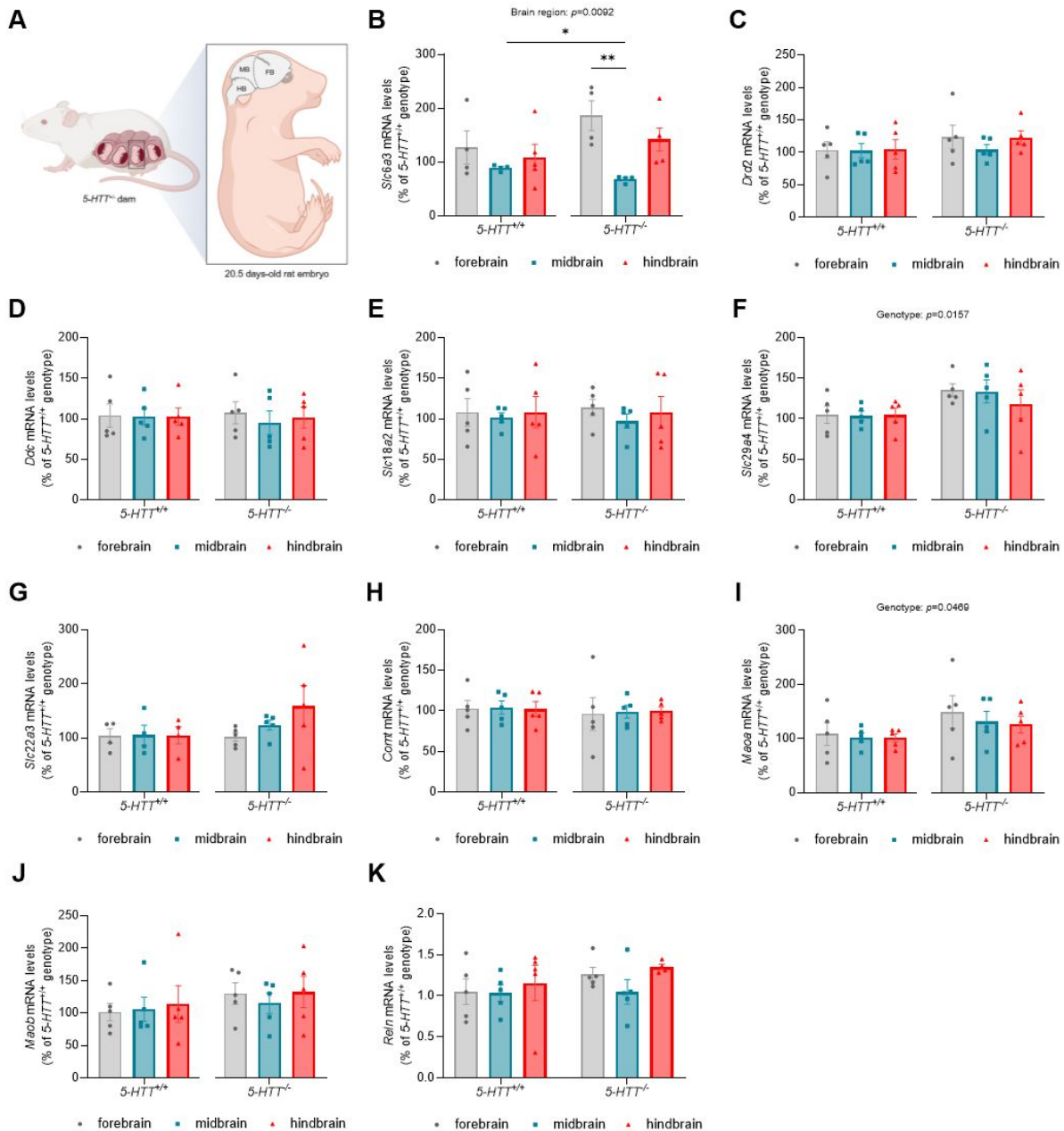
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Supplementary Figure 4. Transcriptional expression profiles of monoaminergic genes during prenatal development. (A) Schematic representation of a serotonergic (5-HT) and dopaminergic (DA) synapse, highlighting key transporters, enzymes, and receptors. (B-G) Temporal expression profiles of key monoaminergic genes (*Slc6a3*, *Drd2*, *Ddc*, *Slc22a3*, *Comt*, and *Maob*) in the forebrain and hindbrain, spanning embryonic day (E)14 to postnatal day (P)0, generated from the EMBL-EBI Expression Atlas (RNA-seq dataset E-MTAB-6811). Expression levels are reported as Transcripts Per Million (TPM). 5-HTP: 5-hydroxytryptophan; L-DOPA: L-3,4-dihydroxyphenylalanine; Slc18a2: vesicular monoamine transporter 2 (VMAT2); Slc22a3: extra neuronal monoamine transporter (OCT3); Slc6a3: dopamine transporter (DAT); Slc6a4: serotonin transporter (5-HTT); Slc29a4: plasma membrane monoamine transporter (PMAT); Drd2: dopamine receptor D2.



Supplementary Figure 5. Molecular characterization of monoaminergic markers in *5-HTT*^{-/-} embryos. (A) Schematic representation of the embryonic day (E)20.5 rat brain, indicating the dissected regions: forebrain (FB), midbrain (MB), and hindbrain (HB). (B-K) mRNA expression levels of *Slc6a3*, *Drd2*, *Ddc*, *Slc18a2*, *Slc29a4*, *Slc22a3*, *Comt*, *Maoa*, *Maob*, and *Reln* were determined by Real-Time quantitative Polymerase Chain Reaction (RT-qPCR) in E20.5 *5-HTT*^{-/-} compared to *5-HTT*^{+/+} embryos. Sample size: $n = 8-10$ (4-5 *5-HTT*^{+/+}, 4-5 *5-HTT*^{-/-}). Each data point represents one animal. Data are presented as the mean $\pm$ standard error of the mean (SEM).

Statistical analysis was performed using two-way ANOVA with brain region and genotype as factors. Statistical significance: $*p < 0.05$ for *5-HTT*^{+/+} compared to *5-HTT*^{-/-}. *5-HTT*: serotonin transporter.

Supplementary Tables

Supplementary Table 1A. Statistical analysis of the spatial distribution of monoaminergic innervation and cortical cellular populations across bins of the infralimbic (IL) cortex within the medial prefrontal cortex of *Tph2*^{-/-} rats. Statistical analyses were performed using the appropriate model for each dataset, considering genotype and bin as experimental factors where applicable. Test statistics and corresponding *p*-values for the main effects and interactions are reported.

Dependent variable	Statistical Model	Bin	Genotype	Bin × Genotype
TH ⁺ fiber length/bin area (×10 ³ μm)	Two-way ANOVA	$F_{(1.588, 6.351)} = 35.01, p=0.0005$	$F_{(1, 4)} = 0.02963, p=0.8717$	$F_{(9, 36)} = 1.270, p=0.2862$
Reln ⁺ cells (%)	Two-way ANOVA	$F_{(1.011, 4.043)} = 71.34, p=0.0010$	$F_{(1, 4)} = 3.085, p=0.1539$	$F_{(9, 36)} = 3.366, p=0.0044$
Ctip2 ⁺ cells (%)	Two-way ANOVA	$F_{(2.258, 9.031)} = 52.46, p<0.0001$	$F_{(1, 4)} = 0.3188, p=0.6025$	$F_{(9, 36)} = 0.3634, p=0.9450$
Tbr1 ⁺ cells (%)	Two-way ANOVA	$F_{(1.954, 7.816)} = 100.8, p<0.0001$	$F_{(1, 4)} = 0.1152, p=0.7514$	$F_{(9, 36)} = 0.9280, p=0.5129$

TH: tyrosine hydroxylase; *Tph2*: tryptophan hydroxylase 2; 5-HT: serotonin.

Supplementary Table 1B. Statistical analysis of the spatial distribution of monoaminergic innervation and cortical cellular populations across bins of the prelimbic (PL) cortex within the medial prefrontal cortex of *Tph2*^{-/-} rats. Statistical analyses were performed using the appropriate model for each dataset, considering genotype and bin as experimental factors where applicable. Test statistics and corresponding *p*-values for the main effects and interactions are reported.

Dependent variable	Statistical Model	Bin	Genotype	Bin × Genotype
TH ⁺ fiber length/bin area (×10 ³ μm)	Two-way ANOVA	$F_{(2.135, 8.541)} = 27.73, p=0.0002$	$F_{(1, 4)} = 15.80, p=0.0165$	$F_{(9, 36)} = 1.303, p=0.2694$

Reln ⁺ cells (%)	Two-way ANOVA	$F_{(1.002, 4.007)} = 133.9, p=0.0014$	$F_{(1, 4)} = 0.1320, p=0.7347$	$F_{(9, 36)} = 0.1219, p=0.9989$
Ctip2 ⁺ cells (%)	Two-way ANOVA	$F_{(1.905, 7.621)} = 48.61, p<0.0001$	$F_{(1, 4)} = 0.7979, p=0.4222$	$F_{(9, 36)} = 2.514, p=0.0239$
Tbr1 ⁺ cells (%)	Two-way ANOVA	$F_{(1.546, 6.184)} = 170.0, p<0.0001$	$F_{(1, 4)} = 1.637, p=0.2699$	$F_{(9, 36)} = 6.937, p<0.0001$

TH: tyrosine hydroxylase; *Tph2*: tryptophan hydroxylase 2; 5-HT: serotonin.

Supplementary Table 1C. Statistical analysis of the spatial distribution of monoaminergic innervation and cortical cellular populations across bins of the cingulate (Cg) cortex within the medial prefrontal cortex of *Tph2*^{-/-} rats. Statistical analyses were performed using the appropriate model for each dataset, considering genotype and bin as experimental factors where applicable. Test statistics and corresponding *p*-values for the main effects and interactions are reported.

Dependent variable	Statistical Model	Bin	Genotype	Bin × Genotype
TH ⁺ fiber length/bin area (×10 ³ μm)	Two-way ANOVA	$F_{(1.857, 7.426)} = 68.55, p<0.0001$	$F_{(1, 4)} = 0.3450, p=0.5885$	$F_{(9, 36)} = 1.214, p=0.3172$
Reln ⁺ cells (%)	Two-way ANOVA	$F_{(1.027, 4.108)} = 439.20, p<0.0001$	$F_{(1, 4)} = 2.597, p=0.1824$	$F_{(9, 36)} = 4.688, p=0.0004$
Ctip2 ⁺ cells (%)	Two-way ANOVA	$F_{(1.489, 5.956)} = 53.69, p=0.0002$	$F_{(1, 4)} = 0.2640, p=0.6345$	$F_{(9, 36)} = 1.236, p=0.3040$
Tbr1 ⁺ cells (%)	Two-way ANOVA	$F_{(1.425, 5.700)} = 107.50, p<0.0001$	$F_{(1, 4)} = 1.301, p=0.3177$	$F_{(9, 36)} = 2.499, p=0.0247$

TH: tyrosine hydroxylase; *Tph2*: tryptophan hydroxylase 2; 5-HT: serotonin.

Supplementary Table 2. Significant Šídák's-corrected post hoc comparisons following spatial distribution analyses of monoaminergic innervation and cortical cellular populations across bins within the medial prefrontal cortex of *Tph2*^{-/-} rats. Only statistically significant comparisons are shown and adjusted *p*-values are reported.

Dependent variable	Subregion	<i>Tph2</i> ^{+/+} vs <i>Tph2</i> ^{+/-}	Adjusted <i>p</i> -value
Ctip2 ⁺ cells (%)	Prelimbic	Bin 7	0.0475
Tbr1 ⁺ cells (%)	Cingulate	Bin 10	0.0415

Tph2: tryptophan hydroxylase 2.

Supplementary Table 3A. Overall quantification of monoaminergic innervation and cortical cellular populations in the infralimbic (IL) cortex of *Tph2*^{-/-} rats. Mean ± standard deviation (SD) values are presented for *Tph2*^{+/+} and *Tph2*^{-/-} rats. For each dependent variable, both the value normalized to the cortical swatch area and the total quantified value are reported. Test statistics and corresponding *p*-values are reported.

Dependent variable	<i>Tph2</i> ^{+/+} (mean ± SD)	<i>Tph2</i> ^{-/-} (mean ± SD)	Statistical Model	Adjusted <i>p</i> -value
TH ⁺ fiber length/total area (×10 ³ μm)	54.81 ± 19.88	57.09 ± 11.52	Unpaired <i>t</i> -test	0.8717
Total TH ⁺ fiber length (μm)	308.10 ± 81.75	334.20 ± 45.93	Unpaired <i>t</i> -test	0.6558
Reln ⁺ cells/μm ² (×10 ³)	0.22 ± 0.03	0.16 ± 0.02	Unpaired <i>t</i> -test	0.0451
Reln ⁺ cells (%)	2.70 ± 0.29	1.91 ± 0.23	Unpaired <i>t</i> -test	0.0205
Ctip2 ⁺ cells/μm ² (×10 ³)	0.96 ± 0.25	0.94 ± 0.05	Unpaired <i>t</i> -test	0.9100
Ctip2 ⁺ cells (%)	11.88 ± 3.52	11.39 ± 0.76	Unpaired <i>t</i> -test	0.8247
Tbr1 ⁺ cells/μm ² (×10 ³)	0.94 ± 0.12	0.97 ± 0.06	Unpaired <i>t</i> -test	0.6591

Tbr1 ⁺ cells (%)	11.56 ± 2.02	11.78 ± 0.88	Unpaired <i>t</i> -test	0.8699
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TH: tyrosine hydroxylase; *Tph2*: tryptophan hydroxylase 2; 5-HT: serotonin.

Supplementary Table 3B. Overall quantification of monoaminergic innervation and cortical cellular populations in the prelimbic (PL) cortex of *Tph2*^{-/-} rats. Mean ± standard deviation (SD) values are presented for *Tph2*^{+/+} and *Tph2*^{-/-} rats. For each dependent variable, both the value normalized to the cortical swatch area and the total quantified value are reported. Test statistics and corresponding *p*-values are reported.

Dependent variable	<i>Tph2</i> ^{+/+} (mean ± SD)	<i>Tph2</i> ^{-/-} (mean ± SD)	Statistical Model	Adjusted <i>p</i> -value
TH ⁺ fiber length/total area (×10 ³ μm)	30.30 ± 2.94	41.34 ± 3.81	Unpaired <i>t</i> -test	0.0165
Total TH ⁺ fiber length (μm)	191.90 ± 15.96	264.5 ± 21.11	Unpaired <i>t</i> -test	0.0090
Reln ⁺ cells/μm ² (×10 ³)	0.14 ± 0.01	0.15 ± 0.01	Unpaired <i>t</i> -test	0.5349
Reln ⁺ cells (%)	1.74 ± 0.05	1.81 ± 0.21	Unpaired <i>t</i> -test	0.5506
Ctip2 ⁺ cells/μm ² (×10 ³)	0.85 ± 0.21	1.00 ± 0.05	Unpaired <i>t</i> -test	0.3059
Ctip2 ⁺ cells (%)	10.44 ± 3.08	12.11 ± 0.87	Unpaired <i>t</i> -test	0.4185
Tbr1 ⁺ cells/μm ² (×10 ³)	0.92 ± 0.01	1.00 ± 0.01	Unpaired <i>t</i> -test	0.0008
Tbr1 ⁺ cells (%)	11.19 ± 0.83	12.14 ± 0.40	Unpaired <i>t</i> -test	0.1476

TH: tyrosine hydroxylase; *Tph2*: tryptophan hydroxylase 2; 5-HT: serotonin.

Supplementary Table 3C. Overall quantification of monoaminergic innervation and cortical cellular populations in the cingulate (Cg) cortex of *Tph2*^{-/-} rats. Mean $\pm$ standard deviation (SD) values are presented for *Tph2*^{+/+} and *Tph2*^{-/-} rats. For each dependent variable, both the value normalized to the cortical swatch area and the total quantified value are reported. Test statistics and corresponding *p*-values are reported.

Dependent variable	<i>Tph2</i> ^{+/+} (mean $\pm$ SD)	<i>Tph2</i> ^{-/-} (mean $\pm$ SD)	Statistical Model	Adjusted <i>p</i> -value
TH ⁺ fiber length/total area ($\times 10^3 \mu\text{m}$)	20.96 $\pm$ 4.28	22.75 $\pm$ 3.09	Unpaired <i>t</i> -test	0.5885
Total TH ⁺ fiber length (μm)	198.20 $\pm$ 51.08	231.50 $\pm$ 37.96	Unpaired <i>t</i> -test	0.4162
Reln ⁺ cells/ μm^2 ($\times 10^3$)	0.11 $\pm$ 0.01	0.11 $\pm$ 0.00	Unpaired <i>t</i> -test	0.9441
Reln ⁺ cells (%)	1.41 $\pm$ 0.06	1.46 $\pm$ 0.07	Unpaired <i>t</i> -test	0.4109
Ctip2 ⁺ cells/ μm^2 ($\times 10^3$)	1.07 $\pm$ 0.31	0.96 $\pm$ 0.11	Unpaired <i>t</i> -test	0.5932
Ctip2 ⁺ cells (%)	14.16 $\pm$ 3.06	13.20 $\pm$ 1.58	Unpaired <i>t</i> -test	0.6541
Tbr1 ⁺ cells/ μm^2 ($\times 10^3$)	1.15 $\pm$ 0.09	1.06 $\pm$ 0.14	Unpaired <i>t</i> -test	0.3884
Tbr1 ⁺ cells (%)	15.43 $\pm$ 0.39	14.52 $\pm$ 1.37	Unpaired <i>t</i> -test	0.3307

TH: tyrosine hydroxylase; *Tph2*: tryptophan hydroxylase 2; 5-HT: serotonin.

Supplementary Table 4A. Statistical analysis of the spatial distribution of monoaminergic innervation and cortical cellular populations across bins of the infralimbic (IL) cortex within the medial prefrontal cortex of *5-HTT*^{-/-} rats. Statistical analyses were performed using the appropriate model for each dataset, considering genotype and bin as experimental factors where applicable. Test statistics and corresponding *p*-values for the main effects and interactions are reported.

Dependent variable	Statistical Model	Bin	Genotype	Bin × Genotype
5-HT ⁺ fiber length/bin area (×10 ³ μm)	Two-way ANOVA	$F_{(2.145, 8.581)} = 46.02, p < 0.0001$	$F_{(1, 4)} = 0.03177, p = 0.8672$	$F_{(9, 36)} = 0.3714, p = 0.9413$
TH ⁺ fiber length/bin area (×10 ³ μm)	Two-way ANOVA	$F_{(2.105, 8.422)} = 9.770, p = 0.0060$	$F_{(1, 4)} = 1.393, p = 0.3033$	$F_{(9, 36)} = 1.512, p = 0.1810$
Reln ⁺ cells (%)	Two-way ANOVA	$F_{(1.027, 4.108)} = 133.9, p = 0.0003$	$F_{(1, 4)} = 0.6374, p = 0.4694$	$F_{(9, 36)} = 0.7136, p = 0.6928$
Ctip2 ⁺ cells (%)	Two-way ANOVA	$F_{(1.916, 7.666)} = 36.98, p = 0.0001$	$F_{(1, 4)} = 1.787, p = 0.2523$	$F_{(9, 36)} = 1.186, p = 0.3333$
Tbr1 ⁺ cells (%)	Two-way ANOVA	$F_{(1.425, 5.700)} = 21.61, p = 0.0029$	$F_{(1, 4)} = 3.129, p = 0.1516$	$F_{(9, 36)} = 0.9100, p = 0.5273$

TH: tyrosine hydroxylase; 5-HT: serotonin; *5-HTT*: serotonin transporter.

Supplementary Table 4B. Statistical analysis of the spatial distribution of monoaminergic innervation and cortical cellular populations across bins of the prelimbic (PL) cortex within the medial prefrontal cortex of *5-HTT*^{-/-} rats. Statistical analyses were performed using the appropriate model for each dataset, considering genotype and bin as experimental factors where applicable. Test statistics and corresponding *p*-values for the main effects and interactions are reported.

Dependent variable	Statistical Model	Bin	Genotype	Bin × Genotype
5-HT ⁺ fiber length/bin area (×10 ³ μm)	Two-way ANOVA	$F_{(1.707, 6.830)} = 20.94, p = 0.0015$	$F_{(1, 4)} = 81.34, p = 0.0008$	$F_{(9, 36)} = 1.168, p = 0.3443$
TH ⁺ fiber length/bin area (×10 ³ μm)	Two-way ANOVA	$F_{(1.542, 6.170)} = 6.155, p = 0.0385$	$F_{(1, 4)} = 3.935, p = 0.1183$	$F_{(9, 36)} = 0.3301, p = 0.9592$
Reln ⁺ cells (%)	Two-way ANOVA	$F_{(1.013, 4.054)} = 383.9, p < 0.0001$	$F_{(1, 4)} = 4.653, p = 0.0972$	$F_{(9, 36)} = 5.537, p < 0.0001$
Ctip2 ⁺ cells (%)	Two-way ANOVA	$F_{(1.582, 6.326)} = 60.53, p < 0.0001$	$F_{(1, 4)} = 5.325, p = 0.0822$	$F_{(9, 36)} = 2.989, p = 0.0092$

Tbr1 ⁺ cells (%)	Two-way ANOVA	$F_{(1,602, 6.410)} = 36.45, p=0.0010$	$F_{(1, 4)} = 6.058, p=0.0696$	$F_{(9, 36)} = 4.154, p=0.0010$
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5-HT: serotonin; TH: tyrosine hydroxylase; 5-HTT: serotonin transporter.

Supplementary Table 4C. Statistical analysis of the spatial distribution of monoaminergic innervation and cortical cellular populations across bins of the cingulate (Cg) cortex within the medial prefrontal cortex of 5-HTT^{-/-} rats. Statistical analyses were performed using the appropriate model for each dataset, considering genotype and bin as experimental factors where applicable. Test statistics and corresponding *p*-values for the main effects and interactions are reported.

Dependent variable	Statistical Model	Bin	Genotype	Bin × Genotype
5-HT ⁺ fiber length/bin area (×10 ³ μm)	Two-way ANOVA	$F_{(1,905, 7.620)} = 34.73, p=0.0002$	$F_{(1, 4)} = 1.474, p=0.2914$	$F_{(9, 36)} = 0.5129, p=0.8554$
TH ⁺ fiber length/bin area (×10 ³ μm)	Two-way ANOVA	$F_{(2,779, 11.12)} = 3.978, p=0.0399$	$F_{(1, 4)} = 1.258, p=0.3248$	$F_{(9, 36)} = 0.5988, p=0.7894$
ReIn ⁺ cells (%)	Two-way ANOVA	$F_{(1,023, 4.090)} = 204.2, p=0.0001$	$F_{(1, 4)} = 0.02607, p=0.8796$	$F_{(9, 36)} = 0.01026, p>0.9999$
Ctip2 ⁺ cells (%)	Two-way ANOVA	$F_{(2,037, 8.146)} = 106.7, p<0.0001$	$F_{(1, 4)} = 0.2485, p=0.6443$	$F_{(9, 36)} = 0.7718, p=0.6428$
Tbr1 ⁺ cells (%)	Two-way ANOVA	$F_{(2,122, 8.489)} = 58.26, p<0.0001$	$F_{(1, 4)} = 2.260, p=0.2072$	$F_{(9, 36)} = 3.251, p=0.0055$

5-HT: serotonin; TH: tyrosine hydroxylase; 5-HTT: serotonin transporter.

Supplementary Table 5. Significant Šídák's-corrected post hoc comparisons following spatial distribution analyses of monoaminergic innervation and cortical cellular populations across bins within the medial prefrontal cortex of 5-HTT^{-/-} rats. Only statistically significant comparisons are shown and adjusted *p*-values are reported.

Dependent variable	Subregion	5-HTT ^{+/+} vs 5-HTT ^{+/-}	Adjusted <i>p</i> -value
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5-HT ⁺ fiber length/bin area (×10 ³ μm)	Infralimbic	Bin 2	0.0355
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Supplementary Table 6A. Overall quantification of monoaminergic innervation and cortical cellular populations in the infralimbic (IL) cortex of *Tph2*^{-/-} rats. Mean ± standard deviation (SD) values are presented for *5-HTT*^{+/+} and *5-HTT*^{-/-} rats. For each dependent variable, both the value normalized to the cortical swatch area and the total quantified value are reported. Test statistics and corresponding *p*-values are reported.

Dependent variable	<i>5-HTT</i>^{+/+} (mean ± SD)	<i>5-HTT</i>^{-/-} (mean ± SD)	<i>Statistical Model</i>	<i>Adjusted p-value</i>
5-HT ⁺ fiber length/total area (×10 ³ μm)	21.42 ± 3.28	21.75 ± 0.77	Unpaired <i>t</i> -test	0.8709
Total 5-HT ⁺ fiber length (μm)	834.40 ± 170.10	713.40 ± 74.41	Unpaired <i>t</i> -test	0.3218
TH ⁺ fiber length/total area (×10 ³ μm)	21.42 ± 3.28	21.75 ± 0.77	Unpaired <i>t</i> -test	0.3033
Total TH ⁺ fiber length (μm)	243.20 ± 81.88	391.50 ± 148.40	Unpaired <i>t</i> -test	0.2041
Reln ⁺ cells/μm ² (×10 ³)	0.41 ± 0.04	0.41 ± 0.08	Unpaired <i>t</i> -test	0.9277
Reln ⁺ cells (%)	3.61 ± 0.95	3.43 ± 0.52	Unpaired <i>t</i> -test	0.7931
Ctip2 ⁺ cells/μm ² (×10 ³)	1.53 ± 0.08	1.95 ± 0.25	Unpaired <i>t</i> -test	0.0549
Ctip2 ⁺ cells (%)	13.56 ± 2.99	16.41 ± 2.14	Unpaired <i>t</i> -test	0.2498
Tbr1 ⁺ cells/μm ² (×10 ³)	1.32 ± 0.15	1.73 ± 0.35	Unpaired <i>t</i> -test	0.1374
Tbr1 ⁺ cells (%)	11.52 ± 0.61	14.55 ± 2.83	Unpaired <i>t</i> -test	0.1431

TH: tyrosine hydroxylase; 5-HT: serotonin; *5-HTT*: serotonin transporter.

Supplementary Table 6B. Overall quantification of monoaminergic innervation and cortical cellular populations in the prelimbic (PL) cortex of *Tph2*^{-/-} rats. Mean ± standard deviation (SD) values are presented for *5-HTT*^{+/+} and *5-HTT*^{-/-} rats. For each dependent variable, both the value normalized to the cortical swatch area and the total quantified value are reported. Test statistics and corresponding *p*-values are reported.

Dependent variable	<i>5-HTT</i>^{+/+} (mean ± SD)	<i>5-HTT</i>^{-/-} (mean ± SD)	<i>Statistical Model</i>	<i>Adjusted p-value</i>
5-HT ⁺ fiber length/total area (×10 ³ μm)	14.92 ± 0.25	16.71 ± 0.25	Unpaired <i>t</i> -test	0.0009
Total 5-HT ⁺ fiber length (μm)	571.80 ± 57.11	552.00 ± 42.51	Unpaired <i>t</i> -test	0.6552
TH ⁺ fiber length/total area (×10 ³ μm)	26.27 ± 8.86	40.21 ± 3.70	Unpaired <i>t</i> -test	0.0658
Total TH ⁺ fiber length (μm)	132.30 ± 58.72	262.90 ± 137.70	Unpaired <i>t</i> -test	0.2053
Reln ⁺ cells/μm ² (×10 ³)	0.32 ± 0.00	0.32 ± 0.00	Unpaired <i>t</i> -test	0.2335
Reln ⁺ cells (%)	2.74 ± 0.29	2.90 ± 0.34	Unpaired <i>t</i> -test	0.5720
Ctip2 ⁺ cells/μm ² (×10 ³)	1.46 ± 0.17	1.56 ± 0.07	Unpaired <i>t</i> -test	0.4011
Ctip2 ⁺ cells (%)	12.34 ± 1.45	14.27 ± 2.19	Unpaired <i>t</i> -test	0.2720
Tbr1 ⁺ cells/μm ² (×10 ³)	1.00 ± 0.11	1.30 ± 0.15	Unpaired <i>t</i> -test	0.0556
Tbr1 ⁺ cells (%)	8.54 ± 0.98	11.98 ± 2.82	Unpaired <i>t</i> -test	0.1162

TH: tyrosine hydroxylase; 5-HT: serotonin; *5-HTT*: serotonin transporter.

Supplementary Table 6C. Overall quantification of monoaminergic innervation and cortical cellular populations in the cingulate (Cg) cortex of *Tph2*^{-/-} rats. Mean $\pm$ standard deviation (SD) values are presented for *5-HTT*^{+/+} and *5-HTT*^{-/-} rats. For each dependent variable, both the value normalized to the cortical swatch area and the total quantified value are reported. Test statistics and corresponding *p*-values are reported.

Dependent variable	<i>5-HTT</i> ^{+/+} (mean $\pm$ SD)	<i>5-HTT</i> ^{-/-} (mean $\pm$ SD)	Statistical Model	Adjusted <i>p</i> -value
5-HT ⁺ fiber length/total area ($\times 10^3 \mu\text{m}$)	1.83 $\pm$ 0.57	2.85 $\pm$ 1.18	Unpaired <i>t</i> -test	0.2525
Total 5-HT ⁺ fiber length (μm)	107.10 $\pm$ 7.41	129.20 $\pm$ 43.70	Unpaired <i>t</i> -test	0.4365
TH ⁺ fiber length/total area ($\times 10^3 \mu\text{m}$)	13.88 $\pm$ 2.77	16.86 $\pm$ 4.47	Unpaired <i>t</i> -test	0.3823
Total TH ⁺ fiber length (μm)	75.84 $\pm$ 8.16	108.70 $\pm$ 63.91	Unpaired <i>t</i> -test	0.4273
Reln ⁺ cells/ μm^2 ($\times 10^3$)	0.20 $\pm$ 0.04	0.21 $\pm$ 0.03	Unpaired <i>t</i> -test	0.7306
Reln ⁺ cells (%)	2.07 $\pm$ 0.24	2.16 $\pm$ 0.07	Unpaired <i>t</i> -test	0.5533
Ctip2 ⁺ cells/ μm^2 ($\times 10^3$)	1.65 $\pm$ 0.22	1.50 $\pm$ 0.10	Unpaired <i>t</i> -test	0.3469
Ctip2 ⁺ cells (%)	17.12 $\pm$ 2.06	15.43 $\pm$ 1.78	Unpaired <i>t</i> -test	0.3429
Tbr1 ⁺ cells/ μm^2 ($\times 10^3$)	1.02 $\pm$ 0.37	1.30 $\pm$ 0.16	Unpaired <i>t</i> -test	0.3015
Tbr1 ⁺ cells (%)	10.79 $\pm$ 4.24	13.38 $\pm$ 2.79	Unpaired <i>t</i> -test	0.4260

TH: tyrosine hydroxylase; 5-HT: serotonin; *5-HTT*: serotonin transporter.

Supplementary Table 7A. Statistical analysis of relative monoaminergic gene expression in 5-HTT^{-/-} rats. Statistical analyses were performed using the appropriate model for each dataset, considering brain region and genotype as experimental factors. Test statistics and corresponding *p*-values for the main effects and interactions are reported.

Dependent variable	Statistical Model	Brain region	Genotype	Brain region × Genotype
<i>Slc6a3</i>	Two-way ANOVA	$F_{(2, 20)} = 5.987, p=0.0092$	$F_{(1, 20)} = 1.746, p=0.2012$	$F_{(2, 20)} = 1.609, p=0.2250$
<i>Drd2</i>	Two-way ANOVA	$F_{(2, 24)} = 0.410, p=0.6681$	$F_{(1, 24)} = 1.569, p=0.2224$	$F_{(2, 24)} = 0.294, p=0.7483$
<i>Ddc</i>	Two-way ANOVA	$F_{(2, 24)} = 0.139, p=0.8713$	$F_{(1, 24)} = 0.027, p=0.8720$	$F_{(2, 24)} = 0.086, p=0.9181$
<i>Slc18a2</i>	Two-way ANOVA	$F_{(2, 24)} = 0.347, p=0.7104$	$F_{(1, 24)} = 0.001, p=0.9768$	$F_{(2, 24)} = 0.064, p=0.9379$
<i>Slc29a4</i>	Two-way ANOVA	$F_{(2, 24)} = 0.321, p=0.7282$	$F_{(1, 8)} = 6.764, p=0.0157$	$F_{(2, 24)} = 0.355, p=0.7051$
<i>Slc22a3</i>	Two-way ANOVA	$F_{(2, 21)} = 0.990, p=0.3883$	$F_{(1, 21)} = 1.934, p=0.1789$	$F_{(2, 21)} = 0.9359, p=0.4080$
<i>Comt</i>	Two-way ANOVA	$F_{(2, 24)} = 0.019, p=0.9816$	$F_{(1, 24)} = 0.269, p=0.6086$	$F_{(2, 24)} = 0.018, p=0.9826$
<i>Maoa</i>	Two-way ANOVA	$F_{(2, 24)} = 0.373, p=0.6924$	$F_{(1, 24)} = 4.391, p=0.0469$	$F_{(2, 24)} = 0.104, p=0.9019$
<i>Maob</i>	Two-way ANOVA	$F_{(2, 24)} = 0.199, p=0.8211$	$F_{(1, 24)} = 1.322, p=0.2616$	$F_{(2, 24)} = 0.112, p=0.8946$
<i>Reln</i>	Two-way ANOVA	$F_{(2, 23)} = 1.867, p=0.1773$	$F_{(1, 23)} = 0.068, p=0.7970$	$F_{(2, 23)} = 0.932, p=0.4083$

Supplementary Table 8A. RT-qPCR analysis of gene expression in the forebrain of 5-HTT^{-/-} rats. Relative gene expression levels are presented as mean ± SD for 5-HTT^{+/+} and 5-HTT^{-/-} rats. Test statistics and corresponding *p*-values are reported.

Gene	5-HTT^{+/+} (mean ± SD)	5-HTT^{+/+} (mean ± SD)	Statistical Model	Adjusted <i>p</i>-value
<i>Slc6a3</i>	127.30 ± 62.06	186.40 ± 55.18	Unpaired <i>t</i> -test	0.2043
<i>Drd2</i>	103.90 ± 28.45	123.70 ± 40.81	Unpaired <i>t</i> -test	0.3979
<i>Ddc</i>	104.00 ± 31.96	107.40 ± 30.15	Unpaired <i>t</i> -test	0.8702
<i>Slc18a2</i>	108.00 ± 37.95	114.00 ± 22.42	Unpaired <i>t</i> -test	0.7687
<i>Slc29a4</i>	105.10 ± 23.95	135.20 ± 17.84	Unpaired <i>t</i> -test	0.0546
<i>Slc22a3</i>	103.80 ± 26.63	101.90 ± 17.54	Unpaired <i>t</i> -test	0.2654
<i>Comt</i>	102.70 ± 22.70	96.20 ± 45.29	Unpaired <i>t</i> -test	0.7801
<i>Maoa</i>	108.40 ± 46.18	149.40 ± 67.00	Unpaired <i>t</i> -test	0.1932
<i>Maob</i>	101.50 ± 29.71	130.00 ± 36.90	Unpaired <i>t</i> -test	0.6264
<i>Reln</i>	104.90 ± 34.91	126.30 ± 18.44	Unpaired <i>t</i> -test	0.2608

Supplementary Table 8B. RT-qPCR analysis of gene expression in the midbrain of 5-HTT^{-/-} rats. Relative gene expression levels are presented as mean ± SD for 5-HTT^{+/+} and 5-HTT^{-/-} rats. Test statistics and corresponding *p*-values are reported.

Gene	5-HTT^{+/+} (mean ± SD)	5-HTT^{+/+} (mean ± SD)	Statistical Model	Adjusted <i>p</i>-value
<i>Slc6a3</i>	89.08 ± 5.43	68.31 ± 6.09	Mann-Whitney-test	0.0286

<i>Drd2</i>	102.70 ± 24.47	104.50 ± 17.28	Unpaired <i>t</i> -test	0.8942
<i>Ddc</i>	102.60 ± 23.28	95.29 ± 32.36	Unpaired <i>t</i> -test	0.6933
<i>Slc18a2</i>	101.50 ± 13.20	97.18 ± 19.73	Unpaired <i>t</i> -test	0.6946
<i>Slc29a4</i>	103.60 ± 12.88	133.80 ± 31.48	Unpaired <i>t</i> -test	0.0828
<i>Slc22a3</i>	105.10 ± 36.73	123.70 ± 20.89	Unpaired <i>t</i> -test	0.3678
<i>Comt</i>	104.10 ± 17.81	98.90 ± 17.44	Unpaired <i>t</i> -test	0.6532
<i>Maoa</i>	102.30 ± 18.88	131.50 ± 42.07	Unpaired <i>t</i> -test	0.1945
<i>Maob</i>	105.80 ± 41.92	115.20 ± 35.21	Unpaired <i>t</i> -test	0.7106
<i>Reln</i>	103.40 ± 23.36	104.70 ± 33.42	Unpaired <i>t</i> -test	0.9413

Supplementary Table 8C. RT-qPCR analysis of gene expression in the hindbrain of 5-HTT^{-/-} rats. Relative gene expression levels are presented as mean ± SD for 5-HTT^{+/+} and 5-HTT^{-/-} rats. Test statistics and corresponding *p*-values are reported.

Gene	5-HTT^{+/+} (mean ± SD)	5-HTT^{-/-} (mean ± SD)	Statistical Model	Adjusted <i>p</i>-value
<i>Slc6a3</i>	109.30 ± 53.39	142.20 ± 47.94	Unpaired <i>t</i> -test	0.3357
<i>Drd2</i>	104.70 ± 33.25	122.80 ± 23.41	Unpaired <i>t</i> -test	0.3511
<i>Ddc</i>	102.80 ± 23.97	101.60 ± 29.43	Unpaired <i>t</i> -test	0.9476

<i>Slc18a2</i>	108.30 ± 43.33	107.70 ± 44.64	Unpaired <i>t</i> -test	0.9825
<i>Slc29a4</i>	104.90 ± 19.31	118.10 ± 39.60	Unpaired <i>t</i> -test	0.5195
<i>Slc22a3</i>	104.80 ± 31.13	159.20 ± 84.49	Unpaired <i>t</i> -test	0.8982
<i>Comt</i>	102.30 ± 20.41	99.87 ± 11.45	Unpaired <i>t</i> -test	0.8216
<i>Maoa</i>	101.40 ± 17.21	126.10 ± 34.87	Unpaired <i>t</i> -test	0.2925
<i>Maob</i>	113.70 ± 63.65	132.70 ± 54.22	Unpaired <i>t</i> -test	0.2151
<i>Reln</i>	136.70 ± 8.87	122.30 ± 37.70	Unpaired <i>t</i> -test	0.4836