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EXPRESSION OF 5HT-RELATED GENES AFTER PERINATAL TREATMENT WITH 5HT AGONISTS

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Abstract

Serotonin (5HT) is a biologically active amine with diverse roles in the mammalian organism. Developmental alterations in 5HT homeostasis could lead to exposure of the developing brain to non-optimal serotonin concentrations that may result in developmental and behavioral deficits. In order to explore the molecular basis of the effects of developmental disturbances on 5HT metabolism on adult central 5HT homeostasis, observed in our previous studies, we measured changes in gene expression of the neuronal 5HT-regulating proteins in adult animals after perinatal treatment with the immediate 5HT precursor 5-hydroxytryptophan (5HTP, 25 mg/kg), or monoamine oxidase (MAO) inhibitor tranylcypromine (TCP 2 mg/kg), during the period of the most intensive development of 5HT neurons - from gestational day 12 until postnatal day 21. Adult animals were sacrificed and the relative mRNA levels for tryptophan hydroxylase 2, MAO A, MAO B, receptors $5HT_{1A}$ and $5HT_{2A}$, 5HT transporter (5HTT) and vesicular monoamine transporter (VMAT) were determined in the raphe nuclei region and prefrontal cortex using Real-Time Relative qRT-PCR. In comparison to the saline treated animals, treatment with 5HTP caused mild but significant increase in MAO A and MAO B mRNA abundance. TCP-treated animals, besides an increase in mRNA abundance for both MAO genes, displayed significantly increased 5HTT and VMAT2 mRNA levels and significantly decreased $5HT_{1A}$ receptor mRNA levels. Our results suggest that perinatal exposure of rats to 5HTP, and especially TCP, induces long-lasting/permanent changes in the expression of 5HT-regulating genes, that presumably underlie 5HT-related neurochemical and behavioral changes in adult animals.

Keywords

• Serotonin • Tranylcypromine • 5-hydroxytryptophan • mRNA • Rat brain • Perinatal treatment

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1. Introduction

Serotonin (5-hydroxytryptamine, 5HT) is a biologically active amine with diverse roles in the mammalian organism, where it is present both in the brain and peripheral tissues. In the periphery, serotonin serves as a regulator of food intake, gastrointestinal, endocrine and cardiovascular function, and as platelet activator [1]. In the developing brain, serotonin serves as a key regulator of serotonergic outgrowth and maturation of target regions [2]. In the mature brain it acts as a neurotransmitter modulating function and plasticity [3,4]. The blood brain barrier, which is not permeable to 5HT, separates the central and peripheral 5HT-compartments allowing for independent regulation of 5HT homeostases maintained through the action of 5HT-regulating proteins.

Each of the 5HT-regulating proteins in the serotonergic synapse has a homologue in the peripheral compartment. Some are present as isoforms encoded by separate genes. The enzyme tryptophan hydroxylase (TPH) is responsible for the rate-limiting step of serotonin synthesis. TPH1 exists mostly in the enterochromafin cells of the intestinal mucosa [5] and TPH2 in 5HT synthesizing neurons [6]. The vesicular monoamine transporter (VMAT) is responsible for the cellular 5HT accumulation and storage in platelet (VMAT1) or neuronal (VMAT2) vesicles [7]. Other 5HT-elements are encoded by the same genes centrally and peripherally. The serotonin transporter (5HTT) is important for serotonin transport into platelets, and reuptake into the presynaptic neuron [8]. Serotonin receptors 1A ($5HT_{1A}R$) and 2A ($5HT_{2A}R$) act as pre- and post-synaptic regulators of synaptic 5HT action [9], while those present on the cells of the intestinal mucosa may be involved in the regulation of 5HT release from the gut [10]. $5HT_{2A}r$ is also present on the platelet membrane and is involved in the process of aggregation during which 5HT is released from the platelets. In both compartments, 5HT is converted into 5-hydroxyindolacetic acid (5HIAA) through the oxidative deamination catalyzed by the mitochondrial enzyme monoamine oxidase (MAO) [11]. MAO comes in two isoforms: MAO A preferentially oxidizes serotonin and norepinephrine, MAO B phenylethylamine, while dopamine and tyramine represent substrates for both isoenzymes.

During fetal and early postnatal development, the blood brain barrier is not fully formed and serotonin can freely cross from the peripheral compartment into the brain and influence the development of the neuronal 5HT system [12]. In fact, peripheral 5HT levels seem to have a role during pregnancy, as maternal [13] and placental [14] serotonin levels were shown to influence fetal brain development in mice. Therefore, it may be assumed that altered 5HT homeostasis, induced either endogenously (i.e.

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by mutations/polymorphisms of one or more of the 5HT-regulating genes) or exogenously (i.e. by taking 5HT-enhancing agents during pregnancy), could lead to the exposure of the developing brain to non-optimal serotonin concentrations that may result in immediate developmental and later behavioral deficits.

Pharmacological studies on the consequences of perinatal neurotransmitter disbalance can be performed in animal models using three approaches: first, by blocking/stimulating receptors; second, through modulation of the activity of the neurotransmitter's transporter; and third, by interfering with the neurotransmitter's metabolism affecting either its synthesis or degradation. Consequences of the perinatal exposure to excessive serotonin concentrations using the first approach have been thoroughly studied through the developmental hyperserotonemia (DHS) model of autism [15]. The second approach has been widely employed in order to investigate the consequences of perinatal exposure to SSRI [16]. The long-term effects of perinatal alterations in serotonin metabolism have been far less studied. We therefore opted for the third approach using the immediate precursor 5-hydroxytryptophan (5HTP) to facilitate 5HT synthesis, or a MAO inhibitor tranylcypromine (TCP) to impede 5HT degradation, during the period of most intensive development of 5HT neurons [17]. In our previous studies we demonstrated that: a) during treatment, 5HTP significantly elevated 5HT concentrations in the peripheral but not in the central 5HT compartment [18], while its long-term effect (in adult animals) was mild but significant reduction in cortical 5HT levels without any effect on 5HT metabolism [19]; b) during treatment, TCP significantly elevated 5HT levels both, in the brain and the periphery [20], while its long-term effect was a robust decrease in cortical and midbrain 5HT levels accompanied with markedly increased 5HT degradation [19]; and c) animals from both groups displayed reduction in anxiety-like behavior that corresponded with the degree of the reduction in 5HT function [21].

The aim of the present study was to explore molecular changes that underlie the observed neurochemical and behavioral alterations in the treated animals, in order to clarify the effects of developmental disturbances in 5HT metabolism on adult central 5HT homeostasis. For this purpose, Wistar rats were subcutaneously administered either 5HTP (25 mg/kg) or TCP (2 mg/kg), from gestational day (GD) 12 until postnatal day (PND) 21. Adult animals were sacrificed and the relative mRNA levels for TPH2, MAO A, MAO B, $5HT_{1A}R$, $5HT_{2A}R$, 5HTT and VMAT2 were determined in the raphe nuclei region (RNR) - the site of 5HT neuronal cell bodies, and prefrontal cortex (PFC) - the site of 5HT axon terminals, using Real-Time Relative qRT-PCR (RT-qPCR).

2. Experimental Procedures

Wistar rats were bred and raised at the Division of Biology of the Faculty of Science, University of Zagreb, according to the procedure described elsewhere [18,20]. In summary, nulliparous Wistar females from the animal facility of the Croatian Institute for Brain Research (University of Zagreb, Croatia), weighing 230-275 g were mated with males of the same strain and age in a 3:1 ratio. Once gravidity was confirmed, the males were separated, and the females randomly assigned to a "saline", "5HTP", or "TCP" group. Two days before parturition, females were separated and remained singly housed until weaning of the pups (at PND 22). After weaning, animals were kept 3-4 per cage. The animals were housed in polycarbonate cages under 12 h light: 12 h dark conditions at 22 ± 2°C, with free access to rat chow and tap water. All efforts were made to reduce the number of animals used and to minimize animal suffering. The study was approved by the Ethics committee of the University of Zagreb, and was conducted in accordance with the Directive of The European Parliament and of the Council (2010/63/EU) and the Croatian Animal Protection Law ("Narodne Novine", 135/2006).

Pharmacological treatment is thoroughly described elsewhere [18,20]. In short, the experimental groups of pups were treated with either 25 mg/kg of the serotonin precursor 5-hydroxy-L-tryptophan (5HTP, Sigma-Aldrich, St. Louis, MO, USA), 2 mg/kg of the monoamine oxidase inhibitor tranylcypromine (TCP, Sigma-Aldrich, St. Louis, MO, USA) or saline, from GD 12 until birth through subcutaneous injections to pregnant females, and from PND 1 until PND 21 through subcutaneous injections of the same doses.

On PND 70 prefrontal cortex and raphe nuclei region samples were collected from 15 saline (10 males, 5 females), sixteen 5HTP (9 males, 7 females), and 17 TCP (9 males, 8 females) treated rats. After anaesthesia and decapitation, the brains were removed from the skulls and briefly frozen in dry ice. A midbrain region containing serotonergic cell bodies of the dorsal and median raphe nuclei was obtained by a 3 mm thick coronal brain slice (plates 43 and 55 in the rat brain atlas, [22], followed by a 3 mm diameter punch into the mid-brain area. A 4 mm coronal cut was then made at the frontal lobes (plate 11) and cortex (all cortical areas anterior to bregma + 1.7 mm) was peeled off. All samples were placed in microtubes and immediately frozen in liquid nitrogen. Samples were disrupted and homogenized with an ultrasonic homogenizer (Bandelin electronic, Mecklenburg-Vorpommern, Germany) in 500 µL of guanidinium thiocyanate solution. The homogenates were then frozen at -80°C until RNA isolation.

Total RNA was isolated from samples using the phenol-free RNAqueous-4PCR kit (Ambion, Inc., Austin, TX, USA) according to manufactures' instructions. Genomic DNA was removed following the kits DNase treatment. RNA concentration and quality was measured in a spectrophotometer (Biochrome), and assessed through standard agarose gel electrophoresis. From 1.25 µg of total RNA added, mRNA was reversely transcribed using MuLV reverse transcriptase (Applied Biosystems, Foster City, CA, USA) and oligo dT primers (Applied Biosystems, Foster City, CA, USA), following manufacturer's instructions, in a total volume of 25 µL. The performance of the reverse transcription was assessed through PCR with positive intron spanning primers provided in the isolation kit. cDNA was stored at -20°C until further processing.

Relative expression of the genes included in Table 1 was assessed through qRT-PCR using the TaqMan gene expression master mix (Applied Biosystems, Foster City, CA, USA) according to manufacturer's instructions. All reactions were performed in a duplex setup with primer

Table 1. Analyzed genes and their assay ID's.

Gene of interest (GOI)	Predesigned TaqMan gene expression assay ID (FAM):	Probe context Sequence[a]
Tryptophan hydroxylase 2 (TPH2)	Rn00598017_m1	AAACTGGCCACGTGCTATTTCTTCA
Monoamine oxidase A (MAO A)	Rn01430961_m1	TGCCTGCCATCATGGGCTTCATACT
Monoamine oxidase B (MAO B)	Rn00566203_m1	AAGAAGCTCTGCAGCCAGTCCATTA
Serotonin transporter (5HTT)	Rn00564737_m1	GGTGGCCAAAGACGCAGGCCCCAGC
Vesicle monoamine transporter 2 (VMAT2)	Rn00564688_m1	AAGTGGCAGCTGGGCGTTGCTTTCC
5HT1a receptor ($5HT_{1A}R$)	Rn00561409_s1	TAATGGGGCAGTGAGGCAGGGTGAC
5HT2a receptor ($5HT_{2A}R$)	Rn00568473_m1	TCCTGTATGGGTACCGGTGGCCTTT

[a] as reported by vendor

limited rat β-actin (ACTB, VIC labelled, Applied Biosystems) as an endogenous control reference gene. The final volume of 20 µL contained 10 µL of master mix, 1 µL of the primers and probes for the reference gene, 1 µL of the primers and probes for the gene of interest (GOI), 6 µL of nuclease free H_2O and 2 µL of cDNA in the range of 20 to 25 ng per reaction. Each sample was run in duplicate. The qRT-PCR setup in the AB 7300 Real-Time PCR System was two minutes at 50°C and then 10 minutes at 95°C, followed by 40 cycles of 95°C for 15 seconds and 60°C for 60 seconds, according to the manufacturer's instruction manuals. The amplification results were analyzed with the 7300 System SDS v1.4. software (Applied Biosystems, Foster City, CA, USA). The cDNA levels were normalized to the endogenous control and relative differences were calculated according to the relative quantitation method.

Delta Cq data were processed with GraphPad Prism 5 Software (GraphPad Software, Inc., La Jolla, CA, USA). The measured parameters were tested for normality of distributions by the method of Kolmogorov and Smirnov. Normally distributed parameters were compared using one-way analysis of variance (ANOVA) with Dunnett's multiple comparison test, which is specific for the comparison of more groups against a control group. Parameters that were not normally distributed were compared using the non-parametric Kruskal-Wallis method, with Dunn's multiple comparison post hoc test. The level of significance was set to 0.05. Values in the text were expressed as means (M) ± standard error of means (S.E.M.).

3. Results

Relative abundance of mRNAs for serotonin related elements was analyzed in the RNR and PFC of the two experimental groups and compared to a control group. Some samples were lost during processing leaving 36 raphe nuclei region (13 5HTP; 12 TCP; 11 saline) and 40 prefrontal cortex (14 5HTP; 13 TCP; 13 saline) samples for further expression analysis. Reliably measurable levels of mRNAs of all investigated genes were found in both analyzed regions. Using two-way ANOVA, it was established that there is no interaction between gender and treatment. Groups were then analyzed separately for gender and treatment influences allowing for a more reliable *post hoc* analysis. Values of gene expression for the regions and genes of interest did not significantly differ between males and females in the integral sample (data not shown), therefore the groups were analyzed as a whole to assess the influence of the treatment on gene expression.

The effects of treatment on the relative mRNA levels of the analyzed genes in each of the regions are shown in Tables 2 and 3. It should be kept in mind that ΔCq value represents a difference between Cq value of an abundant reference gene and Cq value of a GOI; therefore lower ΔCq values indicate higher gene expression. Regardless of treatment, levels of mRNA for TPH2, 5HTT, VMAT2 and $5HT_{1A}R$ were relatively higher in RNR, mRNA for $5HT_{2A}R$ was more abundant in PFC, while similar concentrations of mRNAs for MAO A and MAO B were found in both regions.

In the raphe nuclei region (Table 2), the treatment had very significant influence on mRNA levels for MAO A (KW = 14.49, p = 0.0007) and MAO B (KW = 13.9, p = 0.001) isoenzymes, due to the significantly increased expression of both genes in the groups treated with 5HT enhancers compared to the control group. A similar trend was observed for mRNA levels of TPH2 and VMAT2, although in this case the influence of treatment was only indicative (KW = 5.27, p = 0.0718 for TPH2, and KW = 5.94; p = 0.0513 for VMAT2). The rest of the genes analyzed ($5HT_{1A}R$, $5HT_{2A}R$ and 5HTT) showed no significant changes in relative mRNA abundance (KW = 1.85, p = 0.40; KW = 1.67, p = 0.43; and KW = 1.8, p = 0.41 respectively).

In the frontal cortex (Table 3), the mRNA abundance was significantly influenced by treatment for 5HTT (KW = 9.31, p = 0.0095) and $5HT_{1A}R$ (KW = 7.24, p = 0.027). *Post hoc* analysis revealed that only TCP treated rats had significantly increased 5HTT expression and significantly decreased $5HT_{1A}R$ expression in comparison with the control group of rats. The influence of treatment on TPH2 mRNA levels was only indicative ($F_{(2,35)}$ = 0.139, p = 0.0737), with a tendency for a higher expression in both experimental groups. The abundance of other mRNAs analyzed (for MAOA, MAOB, VMAT2, and $5HT_{2A}R$) was not influenced by treatment with 5HT enhancers ($F_{(2,37)}$ = 0.1136, p = 0.89; KW = 0.77, p = 0.68; $F_{(2,37)}$ = 0.109, p = 0.119; and KW = 1.73, p = 0.42 respectively).

Table 2. ΔCq values# of genes analyzed in raphe nuclei regions of treated animals.

Treatment	N	TPH2	MAOA	MAOB	5HTT	VMAT2	5HT1aR	5HT2aR
5HTP	13	1.06±0.40	4.68±0.20**	4.51±0.18*	5.85±0.52	4.38±0.36	6.01±0.43	9.77±0.23
saline	11	1.77±0.31	5.88±0.21	5.12±0.13	5.35±0.22	5.20±0.43	5.60±0.22	10.1±0.24
TCP	12	0.81±0.48	4.34±0.26***	4.08±0.22***	5.26±0.47	2.86±0.98*	5.30±0.25	9.89±0.34

#Values are expressed as M ± S.E.M. Lower ΔCq numbers indicate higher expression of the genes.
* p < 0.05; ** p < 0.01; *** p < 0.001; experimental vs. control; Dunns *post hoc* analysis after Kruskal-Wallis test

Table 3. ΔCq values# of genes analyzed in prefrontal cortices of treated animals.

Treatment	N	TPH2	MAOA	MAOB	5HTT	VMAT2	5HT1aR	5HT2aR
5HTP	14	7.04±0.30	4.10±0.18	3.49±0.14	15.0±0.23	12.0±0.26	7.60±0.16	7.26±0.19
saline	13	7.94±0.22	4.07±0.18	3.42±0.18	15.4±0.24	12.6±0.32	7.21±0.16	6.96±0.47
TCP	13	7.29±0.30	4.19±0.21	3.36±0.16	14.0±0.47**	11.7±0.33	7.74±0.15**	7.47±0.35

Values are expressed as M ± S.E.M. Lower ΔCq numbers indicate higher expression of the genes.
** p < 0.01; experimental vs. control; Dunns *post hoc* analysis after Kruskal-Wallis test

4. Discussion

4.1 Choice of treatments

We considered 5HTP to be the most suitable choice for the facilitation of 5HT synthesis for the following reasons. First, it readily crosses the placental barrier [23], which is crucial for the prenatal part of the treatment. Second, unlike tryptophan (Trp) which is involved in other metabolic processes, 5HTP is quantitatively converted to serotonin. Third, it allowed us to bypass the rate-limiting step in the synthesis of serotonin (i.e. the action of TPH).

The choice of the MAO inhibitor was somewhat harder. Although MAO A and MAO B under normal physiological conditions have different substrate affinities, both can catabolize the same compounds and are able to take over when the function of the other is compromised through pharmacological inhibition. Accordingly, more pronounced effects on rat 5HT metabolism were observed after the inhibition of both isoforms than after the sole inhibition of MAO A [24–27]. We therefore opted for tranylcypromine, a non-selective irreversible MAO inhibitor.

In addition, both compounds are widely used in the human population: TCP as an antidepressant for treatment of depression resistant to SSRI, and 5HTP as an over-the-counter dietary supplement acting as a mood enhancer, appetite suppressant or sleep aid. However, long-lasting consequences of perinatal exposure to tranylcypromine have hardly been explored, and even less is known about the long-term effects of 5HTP.

Although it would have been more informative to explore dose dependencies of the induced effects, both substances were administered only in single chosen concentrations due to long duration of treatment, low number of pups in experimental groups, and complexity of experimental design. As described earlier [18,20], doses were determined according to literature data and to the results of our experiments on adult animals. 5HTP was administered in a dose sufficient to raise 5HT concentrations without causing the serotonin syndrome, while the given dose of TCP was expected to effectively block most, but not all of the 5HT degradation.

4.2 Sites of expression of 5HT-regulating genes

There is little data available on the levels of expression of genes coding for the 5HT-regulating elements in various brain regions. We therefore first compared relative mRNA abundance for these genes between the site of 5HT neuronal somas and the site of 5HT neuronal endings in the control group of rats. All of the investigated genes were expressed both in the RNR and PFC. Our findings are in line with the reported detection of mRNA in both regions for 5HTT [28], TPH2 [29], $5HT_{1A}R$ [30], $5HT_{2A}R$ [31], and MAO A [32], suggesting that 5HT-regulating proteins are synthesized both, presynaptically in the neuronal somas, and at the target projection sites after axonal transport of mRNA. The presence of mRNA for MAO B [32] and VMAT2 [33] was previously reported only in the RNR of adult animals, but a reason for these discrepancies might lie in different methodologies used to detect mRNA (*in situ* hybridization vs. qRT-PCR) between the mentioned studies and ours. Differences in regional mRNA abundance for each 5HT-regulating gene might reflect different regulation mechanisms of protein synthesis (local vs. central), possibly related to the physiological role of the 5HT-regulating element.

4.3 The effects of pharmacological treatments on the expression of 5HT-regulating genes

Two different 5HT enhancers interfering with 5HT metabolism were used in this study. The first one was the immediate 5HT precursor, which elevated 5HT levels without acting

directly on the 5HT-regulating proteins, and significantly disturbed 5HT-homeostasis only in the peripheral compartment. Still, this transient hyperserotonemia was sufficient to induce mild but significant increases in MAO A and MAO B mRNA abundance in the RNR of adult animals. Upregulation in MAO gene expression was presumably a reaction to the chronic increase in 5HT concentrations during treatment, and was apparently sufficient to prevent a robust increase in brain 5HT concentrations during development. However, gene expression remained upregulated after the wash-out period, and likely represents a cause of a mild reduction in 5HT levels in the cortices of these animals observed in our previous study [19].

The second 5HT-enhancer inhibited the main enzyme involved in the degradation of 5HT and other monoamines, and significantly disturbed 5HT homeostasis both peripherally and centrally. As expected, treatment with TCP induced more robust changes in the brains of adult animals: besides an increase in mRNA abundance for both MAO genes, they displayed significantly increased levels of 5HTT and VMAT2 mRNA and significantly decreased level of $5HT_{1A}R$ mRNA. While we found no literature data on the influence of chronic treatment with MAO inhibitors on the expression of 5HT-regulating elements in the rat brain, dysregulation in both presynaptic and postsynaptic serotonergic mechanisms was found in mice with inactivated MAO A gene [34,35]. The postsynaptic effects, including downregulation of $5HT_{1A}$ receptors, were comparable to ours, while the presynaptic effects, involving downregulation of VMAT2 and 5HTT, were the opposite. This discrepancy is not unexpected considering differences between permanent MAO inactivation at the gene level (knock-out model) and temporary MAO inactivation at the protein level (our model).

During treatment, TCP presumably induced extensive upregulation of MAO gene expression. Since every new dose of TCP also inhibited newly synthesized MAO proteins, this mechanism was not sufficient to maintain 5HT homeostasis during brain development. The attenuation of the excessive 5HT concentrations was probably attempted through the upregulation of 5HTT and VMAT2 expression, in order to increase removal from the synapse and storage in vesicles, and through the downregulation of post-synaptic $5HT_{1A}$ receptors. Regulation of all of the mentioned genes remained altered at adult age. This seems to underlie the drastically reduced 5HT levels with markedly increased 5HT degradation in the brains of these animals observed in our previous study [19].

Interestingly, a common effect of both treatments seems to be the upregulation in expression of both MAO genes. This finding suggests that: 1) both isoforms might have a role in 5HT degradation - although under physiological conditions 5HT is not a preferential substrate for MAO B, it might become a substrate for this enzyme under conditions of high 5HT concentrations; and 2) upregulation of MAO expression might represent the main or the "first-line" mechanism to fight exposure to chronic excessive 5HT concentrations, regardless of the way of interference with 5HT metabolism. Also, both experimental groups displayed indicative increase in TPH2 gene expression, which could represent an attempt to counterbalance the increase in 5HT degradation in the brains of the treated animals which remained after the wash-out period.

4.4 Possible implications

Disbalance in monoaminergic concentrations during brain development appears to have a significant impact on functions of the mature brain [36]. On one hand, disturbances in 5HT transmission have been suggested as an underlying cause of several behavioral disorders, including autism, alcohol dependence and suicidal behavior [37]. On the other hand, if taken during pregnancy, 5HT-enhancing agents such as antidepressants or drugs of abuse could later on lead to behavioral abnormalities in the offspring. Long-lasting behavioral, cellular and molecular changes have been reported in animal models and children perinatally exposed to selective serotonin reuptake inhibitors, cocaine, amphetamine, MDMA, p-chlorophenylalanine and 5-methoxytryptamine [38–44]. Our study is the first to report significant long-lasting/permanent changes in the expression of 5HT-regulating genes induced by chronic perinatal treatment with 5HTP or TCP, that presumably underlie the respective mild or robust neurochemical and behavioral changes observed in our previous studies [19,21]. The obtained results might have the following implications. First, the fact that developmental disturbances in 5HT synthesis or degradation induced permanent alterations in the central 5HT homeostasis, suggests that genes which regulate 5HT metabolism should be considered as potential candidates in 5HT-related behavioral disorders. Indeed, our findings in autistic subjects indicated that disrupted 5HT homeostasis is related to alterations in 5HT metabolism rather than 5HT uptake [45,46]. Second, the developmental disbalance induced in both 5HT-compartments (i.e. by the TCP treatment) leads to more robust permanent alterations of 5HT homeostasis than the developmental disbalance induced only in the peripheral 5HT-compartment (i.e. by the 5HTP treatment). Still, this transient fetal/neonatal (probably along with maternal) hyperserotonemia was sufficient to induce measurable molecular and behavioral 5HT-related changes, indicating the need for further experiments on animal models, as well as prospective studies in humans, which would thoroughly explore the safety of the use of these two 5HT enhancers by pregnant and lactating women.

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Conflict of interest

The authors declare that they have no conflict of interest.

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