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Alterations in the Serotonin and Dopamine Pathways by Cystathionine Beta Synthase Overexpression in Murine Brain

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Abstract

Cystathionine beta synthase (*CBS*) is one of the 225 genes on chromosome 21 (HSA 21) that are triplicated in persons with trisomy 21 (Down syndrome). Although most triplicate HSA21 genes have their orthologous genes on murine chromosome 16, the murine ortholog of *hCBS* is on murine chromosome 17 and thus is not present in the well-studied Ts65Dn mouse model of trisomy 21. Persons with trisomy 21 (T21) present deficits in neurotransmission and exhibit early brain aging that can partially be explained by monoamine neurotransmitter alterations. We used transgenic mice for the *hCBS* gene, which overexpress the CBS protein in various brain regions, to study if CBS overexpression induces modifications in the monoamine neurotransmitters in the hypothalamus, thalamus, hippocampus, and striatum from transgenic and control female and male mice aged 3–4 months and 11–12 months. Sex, age, and brain area each influenced neurotransmitter levels. Briefly, the serotonin pathway was modified by CBS overexpression in various brain areas in female mice but not in male mice. The dopamine pathway was modified in brain regions according to sex and age. These results may allow us to better understand the role of the transsulfuration pathway and especially CBS overexpression in the metabolism of biogenic amines and the catecholamine catabolism in persons with trisomy 21.

Keywords Trisomy 21 (T21) · Monoamine neurotransmitters · Cystathionine beta synthase (CBS) · Transgenic mice Murine brain tissues

Introduction

Trisomy 21 (T21), which causes Down syndrome (DS), affects around eight million people worldwide and is the most common genetic disorder associated with intellectual disability [1]. DS is most often due to full trisomy 21 and rarely to partial trisomy 21 or mosaicism. Individuals with T21 have cognitive and learning deficits, including delayed speech and language development and impairments in spatial and long-term memory [2]. These deficits are associated with neuroanatomical abnormalities including reduced brain size and weight, lamination delay, and abnormal morphogenesis of dendrites and spines [3–5]. The population with T21 is also at a high risk for Alzheimer's neuropathology, even at young ages, and for dementia by the age of 55–60 [5, 6].

Proper regulation of monoamine neurotransmitters like dopamine (DA), noradrenaline (NA), and serotonin (5-hydroxytryptamine: 5-HT) is essential for brain functions such as embryonic development, neuronal morphology, and synaptic plasticity, leading to appropriate learning and cognition [7–11]. Moreover, 5-HT, DA, and NA may interact during both normal and pathological brain functioning [12, 13]. Monoamine pathways are altered in individuals with T21, detected as reduced levels in serum/plasma, of 5-HT and dopamine-beta-hydroxylase activity responsible for converting DA to NA [14–19], reduced urinary levels of homovanillic acid (HVA), one of the final compounds of the DA pathway [20]. Levels of 5-HT and catecholamines are also reduced in the cerebrospinal fluid [21, 22]. In post-mortem brains from individuals with T21, the levels of 5-HT, NA, and dihydroxyphenylacetic acid (DOPAC) are decreased in various brain areas [23–25] as well as in the fetal frontal cortex [26].

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The neural deficits associated with T21 have been explained either directly or indirectly by the overexpression of some chromosome 21 genes [27, 28]. Our understanding of the brain deficits present in people with T21 has largely been improved at the molecular level by the use of murine models of trisomy, especially the Ts65Dn model containing three copies of 115 murine genes on chromosome 16 that are orthologs to Hsa21 genes [29–32]. Recent murine studies revealed several alterations in monoamine neurotransmitter expression in Ts65Dn mice [33–35], as well as in mice trisomic for 19 of the MMU17 genes (Ts1Cje) orthologous to HSA21 genes [36] and in mice transgenic only for the MMU16 gene *Dyrk1a* [37]. Indeed, the importance of the HSA21 orthologs on mouse chromosome 17, especially the *CBS* gene, has been noted [36, 38].

Cystathionine β-synthase (CBS, EC: 4.2.1.22) is encoded by the *CBS* gene on HSA21 and on proximal MMU17 [39]. This enzyme is involved in the metabolism of sulfur-containing amino acids, leading to the formation of cystathionine and water from homocysteine [40]. It also catalyzes the β-replacement reaction in which cysteine is used in place of serine, resulting in the formation of cystathionine and hydrogen sulfide (H_2S) [41–44]. CBS is detected at higher levels in fibroblasts [45], in plasma [46], and in some specific brain areas of individuals with T21 [47, 48]. Yet, the extent of cellular production of CBS in the brain remains a matter of controversy. In mice, CBS is found in most areas of the brain, but predominantly in the cell bodies and neuronal processes of Purkinje cells and Ammon's horn neurons [49]. It is also accumulated in astrocytes [50] and in glial cells [51]. In human cell lines, CBS is found in astroglia [52] with an increased expression in the astrocytes from persons with T21 and within senile plaques from those with Alzheimer's disease [47].

Hydrogen sulfide, which is predominantly produced by CBS in most areas of the brain [51, 53, 54], is a small membrane-permeable gas molecule and one of the more recently identified members in the gasotransmitter family of signaling molecules [55, 56]. H_2S can protect neurons from oxidative stress [57] and can modulate long-term potentiation (LTP) through activation of NMDA receptors [41, 58]. A potential role of H_2S in regulation of monoamine levels has been shown in the central nervous system of rats [59, 60].

In transgenic mice expressing the human *CBS* gene under the control of its own promoter [61], we detected CBS protein and activity in various brain areas. These mice do not show alteration in motor coordination and balance (rotarod experiments) or in passive avoidance, but exhibit an enhanced hippocampic LTP when compared to WT mice [62]. Dp(17)1Yey mice, which contain 19 genes on MMU17 orthologous to HSA21 (including *CBS*), exhibit no difference on either the Morris Water maze task (MWM) or in a contextual fear condition, but exhibit a small increase in LTP [36] when compared to control mice. In addition, hippocampic LTP could be related to monoamine contents [63], and recent studies in rats confirmed the role of CBS in amygdalar LTP [64].

Thus, here, we evaluated if CBS overexpression modulates the levels of monoamine transmitters. We chose to study the hypothalamus, thalamus, hippocampus, and striatum—brain areas involved in most deficits present in individuals with T21—from both 4–5-month-old mice and 11–12-month-old female and male Tg*hCBS*60.4 transgenic (TG) mice, compared to control (WT) mice. Significant modifications in the serotonin and dopamine pathways were found in the various brain areas with major differences according to sex and age. Our findings could enable us to better dissect how the CBS pathway affects neurotransmitter regulation in human brains.

Materials and Methods

Mice

Experiments were performed on the Tg*hCBS*60.4 transgenic line generated at the Eleanor Roosevelt Institute transgenic mouse facility (Denver, USA) on the FVB/N background (Butler 2006). First- and second-generation hemizygous transgenic 60.4P102D1 mice were obtained as described [62]. These mice were transferred to the animal facility (University Paris-Diderot) and were subsequently backcrossed for eight generations on the C57/Bl6J background. Mice were housed in groups of 4–6 and maintained on a 12:12-h light/dark cycle in a temperature-controlled (21 ± 1 °C) and air-conditioned room. Mice were fed a standard laboratory diet (CRM, Special Diets services, Dietex, France Usine). All experiments were carried out in accordance with the European Communities council directives (86/609/EEC) regarding the care and use of animals for experimental procedures. This study was approved by the Animal Ethical Committee of University Paris Diderot (CEEA-40, approval number CEB-001-2011).

At 2 weeks of age, animals were genotyped by PCR analysis of tail DNA using human-specific primers as described [62]. Hemizygous TG mice were compared to wild-type mice from the same litter in each experiment. Female and male mice were 4–5 months and 11–12 months old.

Tissue Sampling

Brains were harvested from mice that were deprived of food to eliminate the effect of diet on monoamine metabolism but not of drinking water, for 5 h (8:30 am to 1:30 pm) before euthanasia by cervical dislocation. Brains were quickly removed and dissected on ice to separate the various brain areas (hypothalamus, thalamus, hippocampus, and striatum). Tissues were immediately deep frozen in liquid nitrogen and stored at − 80 °C until used. For monoamine measurements, the frozen tissues were weighed and then dissolved in 400 μL of ice-cold solution 0.1 M perchloric acid containing 0.4% ethylenediaminetetraacetic acid (EDTA) (100 mL Milli-Q water qs) and then ground using the TissueLyser II (Qiagen) for 2 min with 1.5-mm beads. The homogenates were centrifuged at 3000 rev/min for 30 min at 4 °C. The supernatants were collected and centrifuged again at 16,400 rev/min for 2 min.

High-performance Liquid Chromatography with Electrochemical Detection

Supernatants (200 μL) from brain region homogenates were used for the high-performance liquid chromatography with electrochemical detection (HPLC-ED) analysis. The HPLC instrumentation comprised a Shimadzu apparatus equipped with a LC20AD pump and a SiL20AC autosampler coupled with a Waters 2465 electrochemical detector. Separations were performed using a 150 × 4.6 mm C18 5 μM Beckman Ultrasphere column equipped with two Phenomenex C18 filters in a security guard system. The mobile phase consisted of 150 mM octane sulfonic acid, 8.2 mM ammonium acetate, and 15% methanol (*v*/*v*) at a pH of 3.8 adjusted with glacial acetic acid, and was filtered through a 0.2-μM membrane filter, degassed before use and pumped at a flow rate of 0.8 mL/min.

Eluates were detected at an oxidation potential of 700 mV versus the reference electrode. The column and the detection cell were housed within the Faraday cage of the electrochemical detector set to 25.5 °C. Samples were placed in the autosampler and kept at 4 °C before injection. Injection volume was 40 μL. This system allowed the detection of noradrenaline (NA), adrenaline (AD), dihydroxyphenylacetic acid (DOPAC), dopamine (DA), 5-hydroxyindole-3-acetic acid (5-HIAA), homovanillic acid (HVA), and serotonin: 5-hydroxytryptamine (5-HT) for which the retention times were 7 min, 10 min, 13.5 min, 16.5 min, 21 min, 32 min, and 42 min, respectively.

Chemicals

The compounds AD, DA, DOPAC, HVA, NA, 5-HT, 5-HIAA, EDTA, disodium salt, perchloric acid, and hydrogen chloride were purchased from Sigma, and ultrapure water was obtained with a Milli-Q system (Millipore, Bedford, MA, USA). Standard solutions of each monoamine or metabolite were prepared at 50 ng/mL in ultrapure water for monoamines and in HCl 0.1 N solution for catecholamines and stored at − 20 °C before use.

Statistical Analysis

Monoamine content was expressed as nanograms per gram wet tissue, given as the mean ± standard error of the mean (SEM). The criterion for significance was set at a p value < 0.05. Differences between genotypes (WT and TG) and sexes (females and males) were analyzed using two-way ANOVA with Bonferroni post-test corrections. Mann-Whitney *U* tests were used to compare WT and TG female and male group mice. The statistical analysis and the various graphs presented here were created with the GraphPad InStat software V5.0.

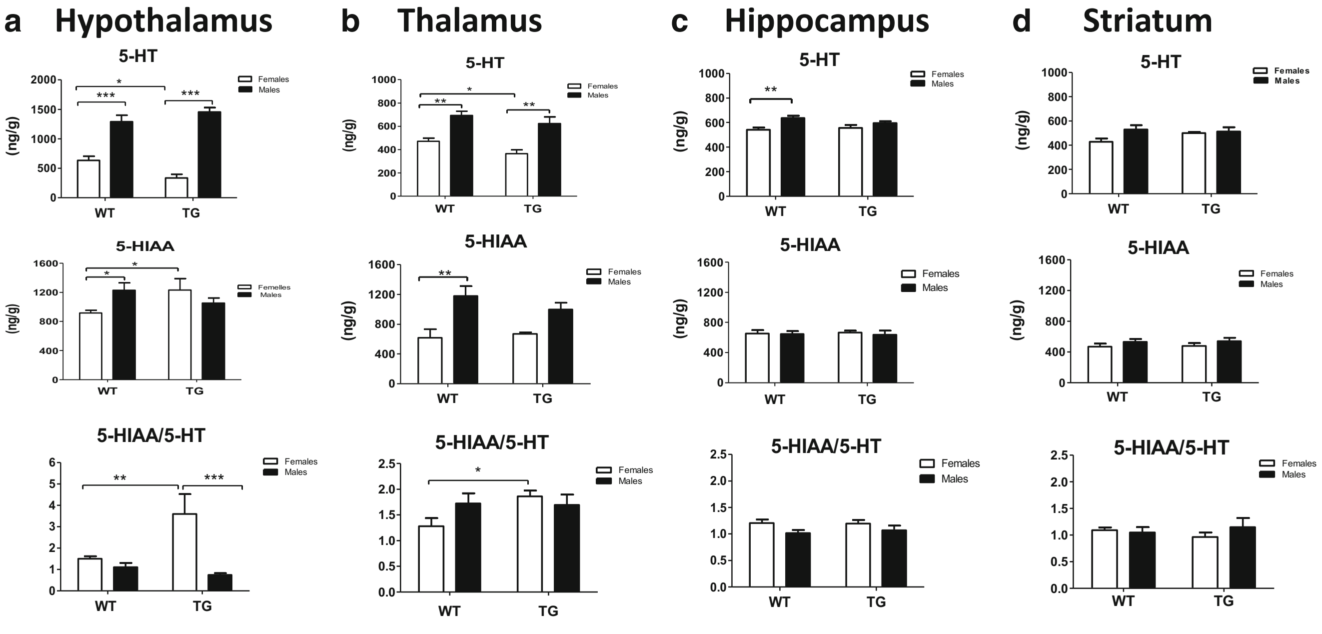

Fig. 1 Serotonin pathway in 4–5-month-old mice. 5-HT content and the ratios 5-HIAA/5-HT are shown in the hypothalamus (**a**), the thalamus (**b**), the hippocampus (**c**), and the striatum (**d**) from female and male control (WT) and transgenic (TG) mice. Control females: $n = 6$, control males: $n = 12$, transgenic females: $n = 4$, transgenic males: $n = 9$. Results are expressed in nanograms per gram wet weight and shown as mean ± SEM for 5-HT and 5-HIAA and as ratios for 5-HIAA/5-HT. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

Correlations were assessed by using Spearman's rank correlation since data were not normally distributed according to the Shapiro-Wilk test. A p value of 0.06–0.10 was considered to indicate a strong statistical tendency due to the sample size.

Results

Effect of CBS Overexpression in 4–5-Month-Old Mice

Serotoninergic Pathway

The 5-HT and 5-HIAA contents and the ratio of 5-HIAA/5-HT, reflecting serotonin metabolism, were measured in the hypothalamus (Fig. 1a), thalamus (Fig. 1b), hippocampus (Fig. 1c), and striatum (Fig. 1d) from 4- to 5-month-old mice of both sexes and genotypes.

In female mice, the 5-HT and 5-HIAA contents were within the same range in the four brain areas from controls. CBS overexpression in female mice induced a 5-HT decrease in the hypothalamus (47%, $p = 0.032$) and the thalamus (22%, $p = 0.046$) and a 5-HIAA increase in the hypothalamus (34%, $p = 0.047$) only. CBS overexpression in female mice also induced increases in the 5-HIAA/5-HT ratio in the hypothalamus (139%, $p = 0.0019$) and in the thalamus (47%, $p = 0.029$).

In male mice of both genotypes, the contents of 5-HT and 5-HIAA and the 5-HIAA/5-HT turnover rate were higher in the hypothalamus than in the other brain areas. CBS overexpression did not induce any significant modification in the four brain areas.

Dopaminergic Pathways

In female mice, the highest contents of DA, DOPAC, and HVA were in the striatum (Fig. 2d) and the lowest in the hippocampus (Fig. 2c) from mice of both genotypes. Moreover, the DA content was higher in hypothalamus (Fig. 2a) than in the thalamus (Fig. 2b) and the hippocampus (Fig. 2c) in female mice of both genotypes. CBS overexpression induced decreases of the DA content in the striatum (31%, $p = 0.05$) and of the DOPAC content in the thalamus (49%, $p = 0.049$) and the hippocampus (37%, $p = 0.048$) but a slight increase in the hypothalamus (20%, $p = 0.05$). HVA could not be measured for technical reasons, except in the striatum, in which the contents were similar from mice of both genotypes. The only modification induced by the CBS overexpression in the DOPAC/DA, HVA/DOPAC, and HVA/DA turnover rates, reflecting DA catabolism, was a slight increase in HVA/DA in the striatum (Fig. 3d).

In male mice, CBS overexpression induced decreases of the DA contents in the hippocampus (30%, $p = 0.046$) and in the

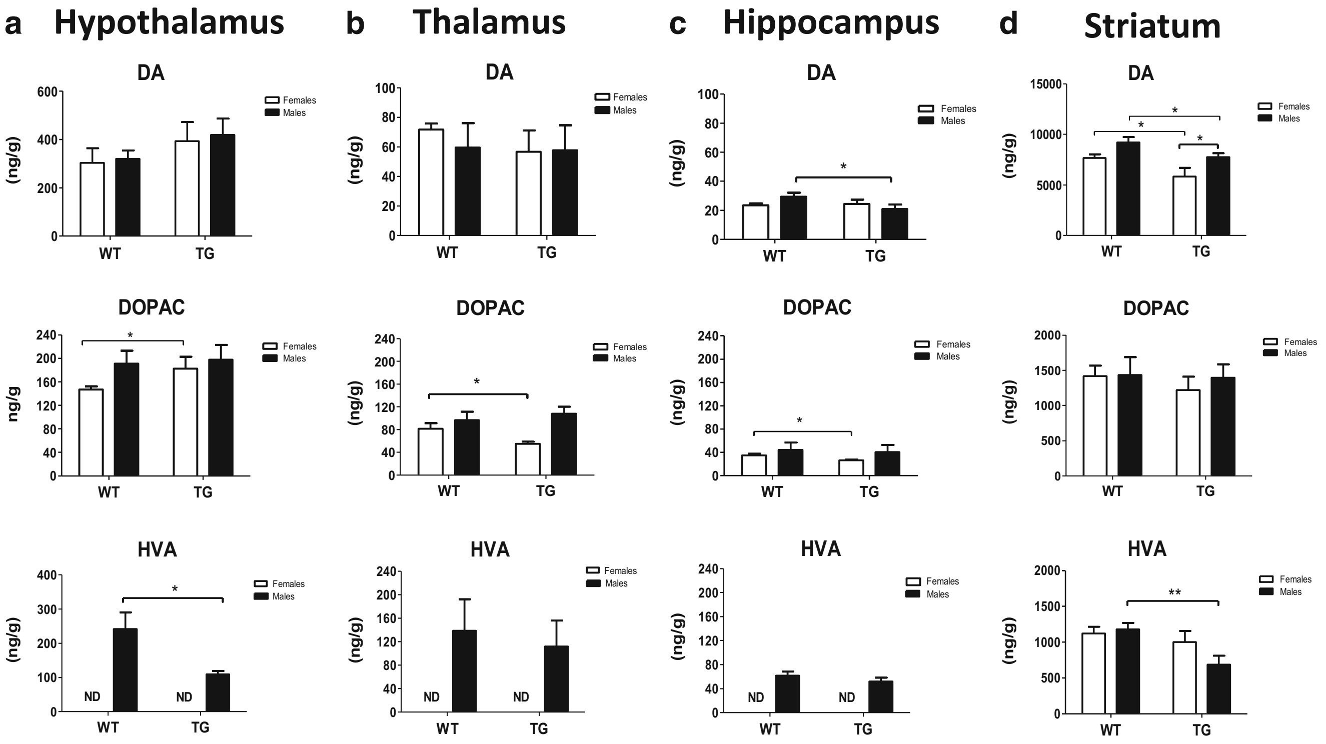

Fig. 2 Dopaminergic pathways in 4–5-month-old mice. The DA, DOPAC, and HVA are shown in the hypothalamus (**a**), the thalamus (**b**), the hippocampus (**c**), and the striatum (**d**) from control (WT) and transgenic (TG) female and male mice. Control females: $n = 6$, control males: $n = 12$, transgenic females: $n = 4$, transgenic males: $n = 9$. Results are expressed in nanograms per gram wet weight and shown as mean ± SEM. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$

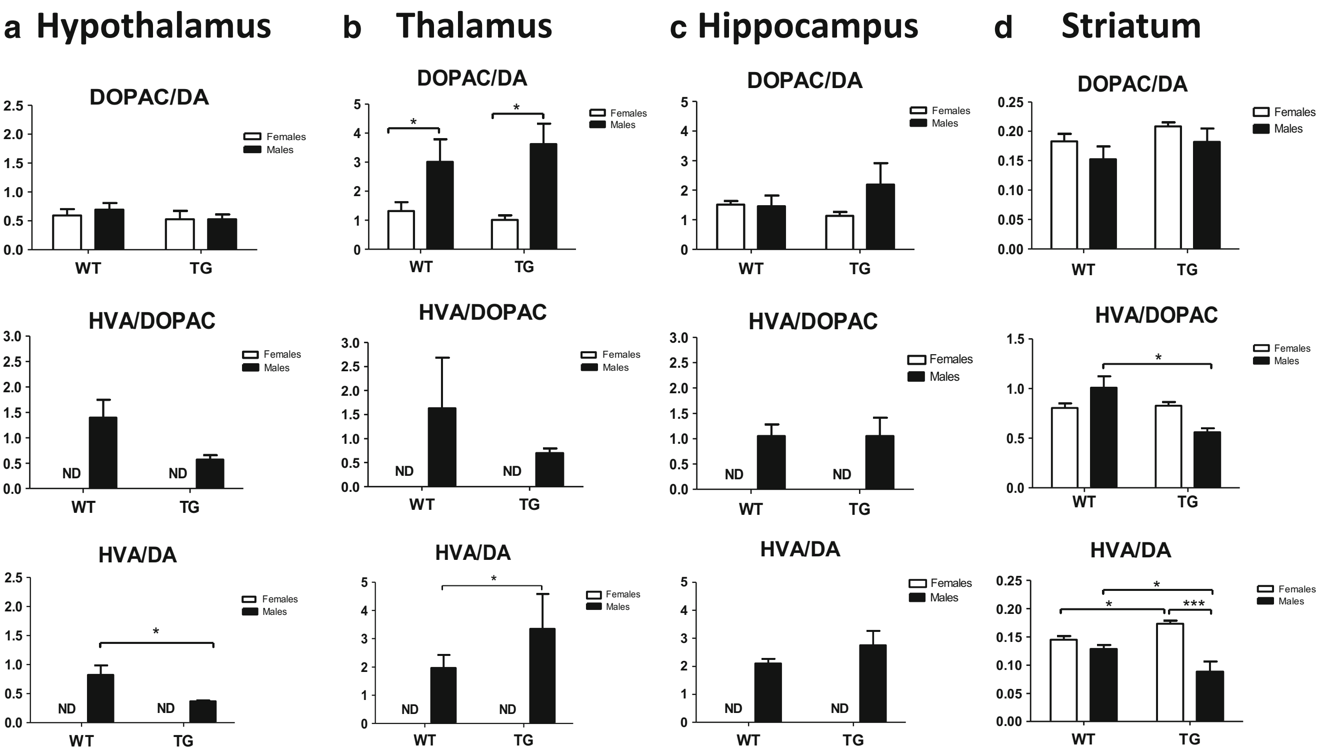

Fig. 3 Dopaminergic pathways in 4–5-month-old mice. DOPAC/DA, HVA/DOPAC, and HVA/DA are shown in the hypothalamus (**a**), the thalamus (**b**), the hippocampus (**c**), and the striatum (**d**) from control (WT) and transgenic (TG) female and male mice. Control females: $n = 6$, control males: $n = 12$, transgenic females: $n = 4$, transgenic males: $n = 9$. Results are expressed as ratios with $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$

striatum (27%, $p = 0.049$) (Fig. 2c, d, respectively) and of the HVA contents in the hypothalamus (55%, $p = 0.0485$) (Fig. 2a) and in the striatum (42%, $p = 0.006$) (Fig. 2d). The HVA/DOPAC and HVA/DA turnover rates were decreased in the striatum (45%, $p = 0.023$ and 39%, $p = 0.030$, respectively) (Fig. 3d). HVA/DA ratios were decreased in the hypothalamus (56%, $p = 0.027$) but increased in the thalamus (71%, $p = 0.050$) (Fig. 3a, b, respectively).

The Noradrenergic Pathways

In female mice, the NA content was the lowest in the striatum (Fig. 4d) and the highest in the hypothalamus and thalamus (Fig. 4a, b, respectively) from WT and TG. No modification was induced by CBS overexpression. For technical reasons, the AD contents were measured in the hypothalamus and thalamus only. CBS overexpression induced a drastic decrease in the AD content in the hypothalamus (250%, $p = 0.050$) (Fig. 4a), but no modification was induced in the NA/DA and AD/NA turnover rates in the four brain areas tested.

In male mice, the NA contents were the lowest in the striatum and the highest in the hypothalamus as in female mice (Fig. 4). CBS overexpression induced increases of the AD content (50%, $p = 0.021$ and of the AD/NA ratio (39%, $p = 0.028$ in the hypothalamus) (Fig. 4a) and an increase in the NA/DA content (43%, $p = 0.005$) in the hippocampus (Fig. 4c)).

Effect of CBS Overexpression in 11–12-Month-Old Mice

Serotoninergic Pathway

The 5-HT and 5-HIAA contents and the 5-HIAA/5-HT ratios were measured in the four brain tissues from 11- to 12-month TG and WT female and male mice of both genotypes (Fig. 5).

In female mice, the 5-HT and 5-HIAA contents were the highest in the hypothalamus and the lowest in the striatum (Fig. 5a, d, respectively). CBS overexpression induced modifications in the thalamus only (Fig. 5b) with a decrease in the 5-HT content (24%, $p = 0.048$) and an increase in the 5-HIAA/5-HT turnover rate (44%, $p = 0.023$).

The male mice had similar profiles to the female mice. Similarly to results in adult male brain areas, CBS overexpression did not induce any modification in the serotonin pathway (Fig. 5a–d).

Dopaminergic Pathways

The DA content, its metabolites DOPAC and HVA, and the DOPAC/DA, HVA/DA, and HVA/DOPAC turnover ratios (Fig. 6) were measured in the four brain areas from male mice of both genotypes, but only in the hypothalamus and striatum

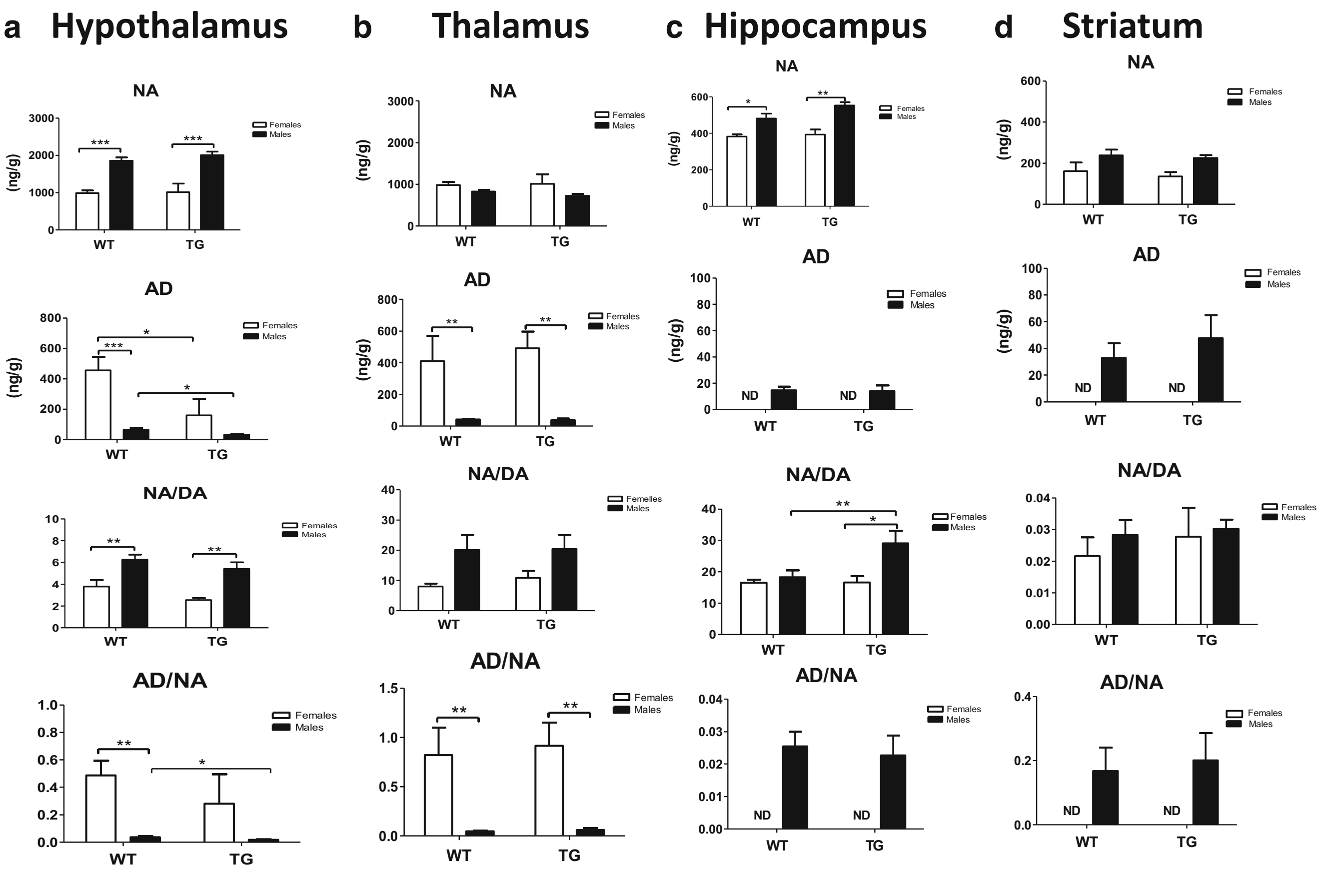

Fig. 4 Noradrenergic pathways in 4–5-month-old mice. NA, AD, NA/DA, and AD/NA are shown in the hypothalamus (**a**), the thalamus (**b**), the hippocampus (**c**), and the striatum (**d**) from control (WT) and transgenic (TG) female and male mice. Control females: $n = 6$, control males: $n = 12$, transgenic females: $n = 4$, transgenic males: $n = 9$. Results are expressed in nanograms per gram wet weight and shown as mean ± SEM for NA and AD and as ratios for NA/DA and AD/NA. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

from female mice of both genotypes (Fig. 6a, b, respectively) for technical reasons.

In female mice, the DA and DOPAC contents were much higher in the striatum than in the hypothalamus (Fig. 6a, b, respectively). The only modification induced by CBS overexpression was in the hypothalamus with an increase in the DA content (48%, $p = 0.012$) and a decrease in the DOPAC/DA ratio (58%, $p = 0.027$) (Fig. 6a).

In male mice, the DA and DOPAC contents were much higher in the striatum than in the three other brain areas (Fig. 6b, a and data not shown). CBS overexpression induced in the striatum a DA content decrease (64%, $p = 0.0007$) and a HVA/DA increase (41%, $p = 0.011$) (Fig. 6b). A decrease in the DOPAC content was also measured in the hippocampus (52%, $p = 0.047$, data not shown). In the hypothalamus, the turnover rates HVA/DOPAC and HVA/DA were decreased (28%, $p = 0.001$ and 29%, $p = 0.047$, respectively) (Fig. 6a).

The Noradrenergic Pathways

In female mice, the lowest contents of NA and AD were detected in the striatum (Fig. 7a–d). CBS overexpression induced in the hypothalamus an increase in the AD content (56%, $p = 0.016$) and a decrease in NA/DA (49%, $p = 0.010$) (Fig. 7a).

In male mice, the NA contents were higher in the hypothalamus and the thalamus than in the two other brain areas (Fig. 7a, b). CBS overexpression did not induce any modification in the noradrenergic pathways in the four brain areas tested (Fig. 7).

Effects of Sex

Serotoninergic Pathway

In adult mice (Fig. 1), the 5-HT contents were higher in the hypothalamus and thalamus from males of both genotypes (Fig. 1a, b) and in the hippocampus from WT males (Fig. 1c). The 5-HIAA contents were higher in hypothalamus and thalamus (Fig. 1a, b) from adult WT male mice and similar in the two other tissues from both sexes (Fig. 1c, d). The 5-HIAA/5-HT turnover rates were higher in TG female than in TG male in the hypothalamus and similar for both sexes in three other tissues.

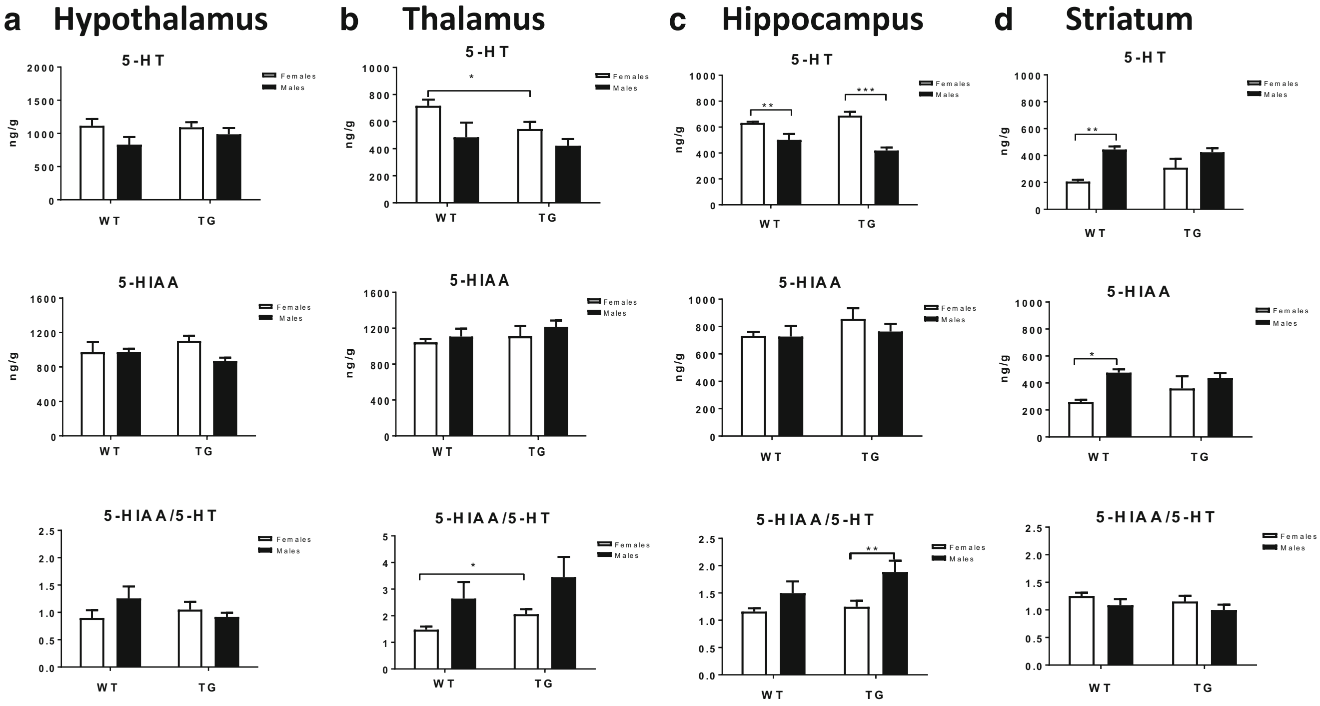

Fig. 5 Serotonin pathway in 11–12-month-old mice. 5-HT content and the ratios 5-HIAA/5-HT are shown in the hypothalamus (**a**), the thalamus (**b**), the hippocampus (**c**), and the striatum (**d**) from female and male control (WT) and transgenic (TG) mice. Control females: $n = 4$, and control males: $n = 4$, transgenic females: $n = 5$, transgenic males: $n = 8$. Results are expressed in nanograms per gram wet weight and shown as mean ± SEM for 5-HT and 5-HIAA and as ratios for 5-HIAA/5-HT. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$

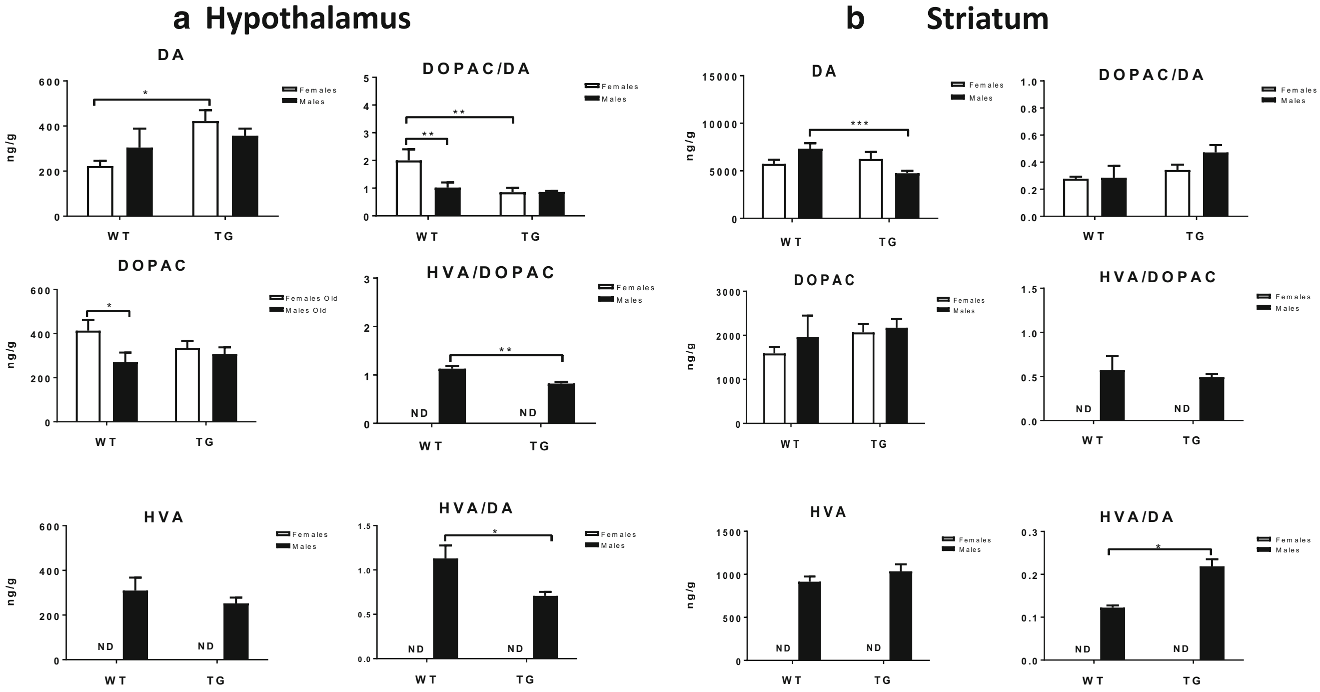

Fig. 6 Dopaminergic pathways in 11–12-month-old mice. The DA, DOPAC, HVA, DOPAC/DA, HVA/DOPAC, and HVA/DA are shown in the hypothalamus (**a**) and the striatum (**b**) from control (WT) and transgenic (TG) female and male mice. Control females: $n = 4$, control males: $n = 4$, transgenic females: $n = 5$, transgenic males: $n = 8$. Results are expressed in nanograms per gram wet weight and shown as mean ± SEM for DA, DOPAC, and HVA and as ratios for DOPAC/DA, HVA/DOPAC, and HVA/DA. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$

In middle-aged mice (Figs. 5a–d), the 5-HT contents were lower in the hippocampus from males of both genotypes and higher in the striatum from WT males when compared to those from females. The 5-HIAA/5-HT turnover rates were similar for both sexes except in the hippocampus for TG mice (Fig. 5c).

Dopaminergic Pathways

In 4–5-month-old mice, the DA contents were higher in the striatum from males of both genotypes (Fig. 2d). No difference according to sex was observed for the DA, DOPAC, and HVA contents in the other brain areas (Fig. 2a–c). The DOPAC/DA turnover rates were higher in the thalamus from male mice of both genotypes in comparison to those from female mice (Fig. 3b).

In 11–12-month-old mice (Fig. 6a, b and data not shown), no difference in the DA contents according to sex was detected in the four areas tested. In the hypothalamus, DOPAC content and DOPAC/DA were lower in WT male mice only (Fig. 6a) and no other modification according to sex was detected (Figs. 6b–d).

Noradrenergic Pathways

In 4–5-month-old mice (Fig. 4), the NA contents were much higher in the hypothalamus and the hippocampus from males of both genotypes, but surprisingly, the AD contents were drastically lower in the hypothalamus from males compared to those from females of both genotypes (Fig. 4a, b). Consequently, the NA/DA ratios were higher in the hypothalamus from male mice of both genotypes and in the hippocampus from TG male mice only, but the AD/NA ratios were dramatically higher in the hypothalamus and thalamus from female mice of both genotypes (Fig. 4a, b).

In 11–12-month-old mice (Fig. 7), the NA contents were higher in the thalamus from males of both genotypes than in females (Fig. 7b) and in the striatum from WT mice (Fig. 7d) but lower in the hippocampus from TG mice only (Fig. 7c). As in adult mice, the AD contents were lower in the thalamus and hippocampus from male mice of both genotypes (Fig. 7b, c) and in the hypothalamus from TG males than in females (Fig. 7a). No modification was detected in the NA/DA ratio, but, as in adult mice, the AD/NA ratios were higher in the thalamus, hippocampus, and striatum from WT female mice of both genotypes compared to those from male mice (Fig. 7b–d).

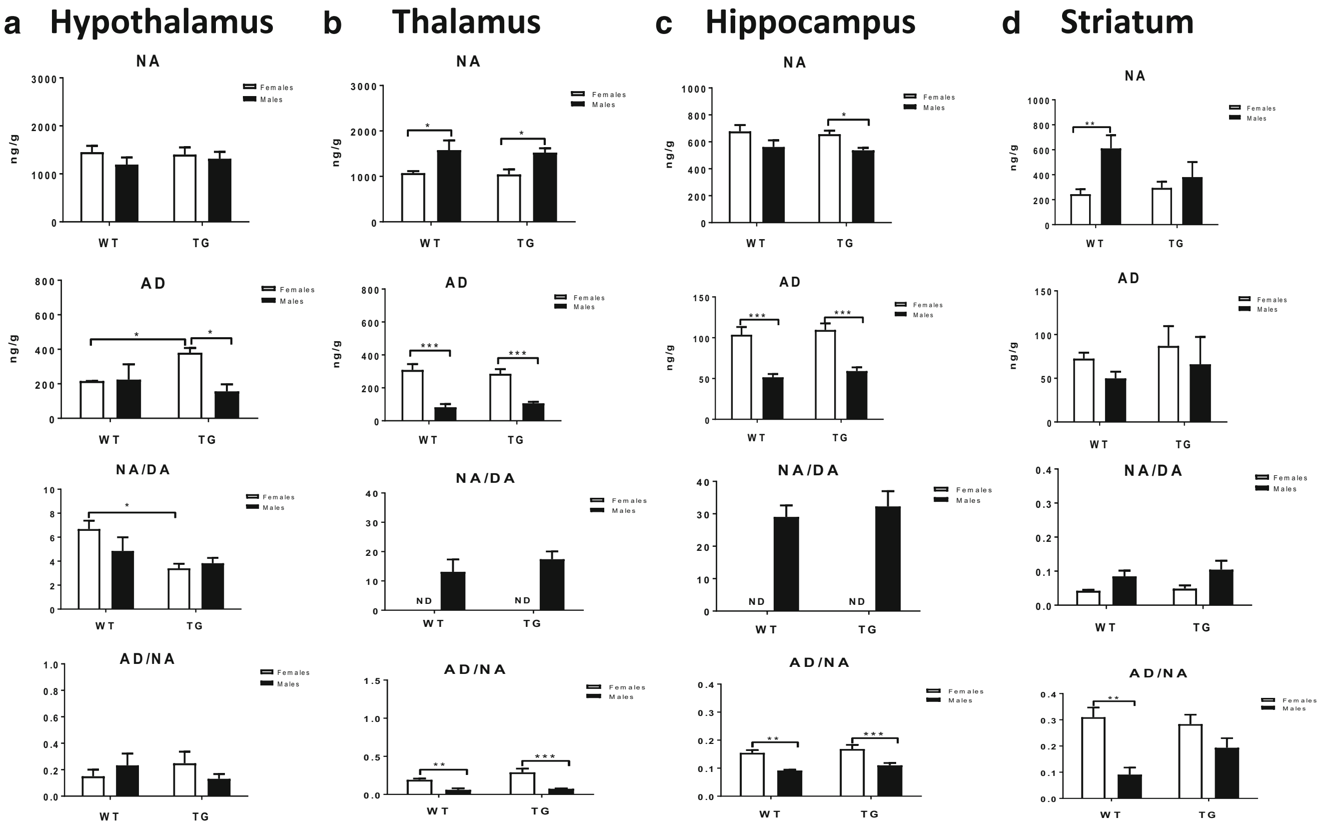

Fig. 7 Noradrenergic pathways in 11–12-month-old mice. NA, AD, NA/DA, and AD/NA are shown in the hypothalamus (**a**), the thalamus (**b**), the hippocampus (**c**), and the striatum (**d**) from control (WT) and transgenic (TG) female and male mice. Control females: $n = 4$, control males: $n = 4$, transgenic females: $n = 5$, transgenic males: $n = 8$. Results are expressed in nanograms per gram wet weight and shown as mean ± SEM for NA and AD and as ratios for NA/DA and AD/NA. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$

Spearman Correlations

Spearman correlation analysis revealed a positive correlation between DA and HVA levels in the striatum of 4–5-month-old adult mice ($r = 0.610$, $p < 0.012$). We also found a trend toward negative correlation between DA and AD in the hypothalamus from 4- to 5-month-old male mice ($r = -0.583$, $p < 0.099$).

Discussion

CBS overexpression induces both a decrease in homocysteine level and an increase in the H_2S neuromodulator, which has been shown to increase neuroplasticity and thus might be a neuroprotector in various conditions [65]. Although the homocysteine level is moderately decreased in T21 as a consequence of CBS overexpression, it is increased in persons with hyperhomocysteinemia, who also present mild cognitive impairment [66]. Moreover, cognitive deficits induced by homocysteine can be attenuated by improving endogenous H_2S levels through catecholamine modulation [67].

The direct consequences on the nervous system of CBS gene overexpression by itself have not been clearly evaluated, but Tg*hCBS* mice have an enhanced LTP when compared to control mice [62]. To better understand if CBS overexpression induces monoamine modulation in the brain, we used the same transgenic mice as in [62] to evaluate by HPLC-EC the monoamine content in the hypothalamus, thalamus, hippocampus, and striatum from mice 4–5 months and 11–12 months old. Important differences in monoamine pathways have been reported according to sex in both rat and mouse models [68–71]. Moreover, although most of the studies on the nervous system using rodent models for T21, especially with partial trisomies, were performed only on male mice, the expressions of some HSA21 proteins likely differ according to sex as well as age [72, 73]. Thus, we performed the present study using both female and male TG and WT mice from two different ages (4–5 and 11–12 months old).

To validate the results obtained in transgenic mice, we first compared the present results obtained in control 4–5-month-old male mice with those previously published. In the 5-HT pathway, our results are in the same range as those previously published, although we detected a higher content of 5-HT and 5-HIAA in the hypothalamus [74–76]. Our results showing the highest DA content in the striatum and the lowest in the hippocampus validate previous results [77]. The NA content was higher in hypothalamus than in the other areas, as previously published [74, 75, 77], and different in female and male [78]. The present results in WT male mice are also in accordance with our prior findings [37].

The effects of CBS overexpression obtained in the present study are summarized in Table 1 and in Fig. 8a, b.

In the Hypothalamus

In the serotoninergic pathway, decreased 5-HT contents were detected in 4–5- and 11–12-month-old female mice, but increased 5-HIAA and 5-HIAA/5-HT in younger females only. The scatter plot for 5-HIAA/5-HT in the 11–12-month-old male mice shows no significant difference between TG and WT mice (Fig. 8bi).

In the dopamine pathway, the main modifications were increased DOPAC in 4–5-month-old female mice and DA in 11–12-month-old female mice. The scatter plot for DA shows no significant difference between TG and WT mice (Fig. 8bii).

In the noradrenergic pathway, although the NA content was not modified, the AD contents were decreased in 4–5-month-old mice from both sexes and increased in 11–12-middle-aged female only.

In the Thalamus

No modification was observed for the three pathways in male mice of both ages. The only modifications were decreased 5-HT content and increased 5-HIAA/5-HT turnover rates and decreased DOPAC content in females of both ages. The scatter plot for NA content shows no significant difference between TG and WT 4–5-month-old male mice (Fig. 8biii).

In the Hippocampus

In the serotoninergic pathway, CBS overexpression did not induce any modification according to sex or age, although an increase with $0.05 < p > 0.08$ only was measured in the NA content from 4- to 5-month female mice (Fig. 8aii).

In the dopamine pathway, although in 4–5-month-old female mice the DA content was not modified, DOPAC was decreased. The main modifications were for adult 4–5-month-old male mice with decreased DA, increased NA, and increased NA/DA. The DA decrease might be related to the LTP increase reported in previous studies [62, 79] and in a recent one on LTP in rats [64].

In the Striatum

No significant modification was shown in the serotonin pathway; nevertheless, an increased 5-HT was observed in 4–5-month-old female mice with $0.05 < p > 0.08$ only (Fig. 8ai). Major modifications in the dopaminergic pathway, especially in male mice from both ages, were detected. The DA decrease in adult 4–5-month-old male mice also induced decreases in the HVA metabolite content and in the turnover HVA/DOPAC and HVA/DA. Indeed, a positive correlation was found between DA and HVA. The DA content was also decreased in adult 4–5-month-old female and

Table 1 Summary of the CBS overexpression effects in the hypothalamus, the thalamus, the hippocampus, and the striatum from 4- to 5- and from 11- to 12-month-old female and male transgenic mice compared to controls. Only significant results are given

	Hypothalamus				Thalamus				Hippocampus				Striatum			
	4-5 months		11-12 months		4-5 months		11-12 months		4-5 months		11-12 months		4-5 months		11-12 months	
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male
	4 TG vs 6 WT	9 TG vs 12 WT	5 TG vs 4 WT	8 TG vs 4 WT	4TG vs 6WT	9 TG vs 12 WT	5 TG vs 4 WT	8 TG vs 4 WT	4 TG vs 6 WT	9 TG vs 12 WT	5 TG vs 4 WT	8 TG vs 4 WT	4 TG vs 6 WT	9 TG vs 12 WT	5 TG vs 4 WT	8 TG vs 4 WT
	Serotoninergic Pathway															
5-HT	* ↘	=	=	=	* ↘	=	* ↘	=	=	=	=	=	=	=	=	=
5-HIAA	* ↗	=	=	=	=	=	=	=	=	=	=	=	=	=	=	=
5-HIAA/5-HT	** ↗	=	=		* ↗	=	* ↗	=	=	=	=	=	=	=	=	=
	Dopamine Pathway															
DA	=	=	* ↗	=	=	=	ND	=	=	* ↘	ND	=	* ↘	* ↘	=	*** ↘
DOPAC	* ↗	=	=	=	* ↘	=	=	=	* ↘	=	=	* ↘	=	=		=
HVA	ND	* ↘	ND	=	ND	=	ND	=	ND	=	ND	=	=	** ↘	ND	=
DOPAC/DA	=	=	** ↘	=	=	=	ND	=	=	=	ND	=	=	=	=	
HVA/DOPAC	ND	=	ND	** ↘	ND	=	ND	=	ND	=	ND	=	=	* ↘	ND	=
HVA/DA	ND	* ↘	ND	* ↘	ND	=	ND	=	ND	=	ND	=	* ↗	* ↘	ND	* ↗
	Noradrenergic Pathway															
DA	=	=	* ↗	=	=	=	ND	=	=	* ↘	ND	=	* ↘	* ↘	=	*** ↘
NA	=	=	=	=	=	=	=	=	=	=	=	=	=	=	=	=
AD	* ↘	* ↘	* ↗	=	=	=	=	=	ND	=	=	=	ND	=	=	=
NA/DA	=	=	* ↘	=	=	=	ND	=	=	** ↗	ND	=	=	=	=	=
AD/NA	=	* ↘	=	=	=	=	=	=	ND	=	=	=	ND	=	=	=

*p<0.05; ** p<0.01; *** p<0.001 ; ND = Non Determined

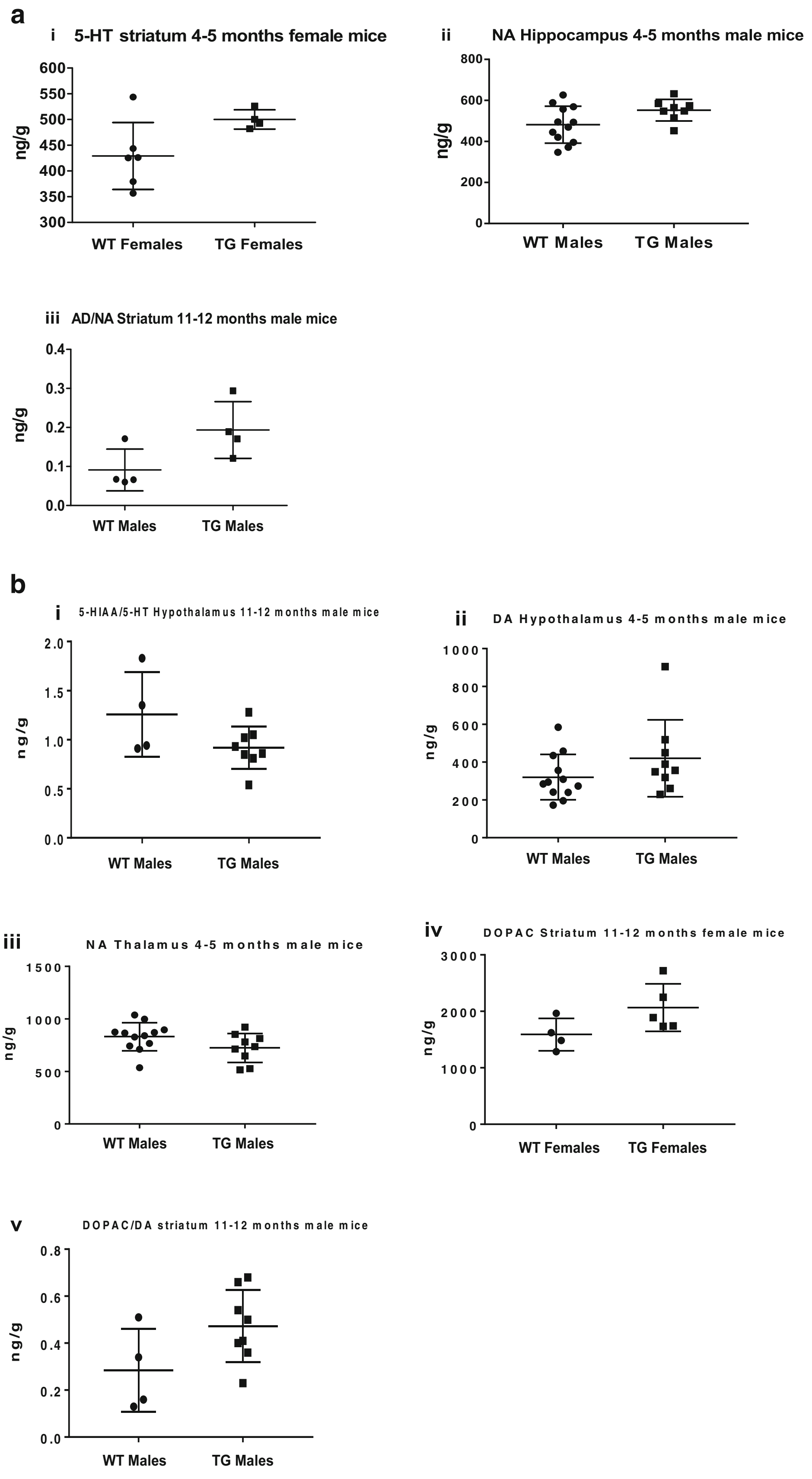

Fig. 8 Scatter plots for Table 1 results that did not reach statistical significance. Increases or decreases almost significant with $0.05 < p > 0.08$ (**ai-iii**). Increases or decreases of more than 25% were observed but were not statistically significant ($p > 0.09$) (**bi v**)

in 11–12-month-old male mice. The scatter plot for DOPAC content shows no significant difference between 11- and 12-month-old TG and WT females (Fig. 8biv); the scatter plot of DOPAC/DA content shows no significant difference between 11- and 12-month-old TG and WT males (Fig. 8bv). Almost significant increased AD/NA was also measured in 11–12-month-old male mice (Fig. 8aiii).

Conclusion

In summary, our results show that CBS overexpression induced drastic effects on the serotoninergic and dopaminergic pathways according to brain area, age, and sex.

Although alterations concerning the metabolism of biogenic amines and catecholamine catabolism in persons with T21 have been reported in many studies, few studies using animal models have been reported. Modulations of neurotransmitters in the two main trisomic mouse models for T21, the Ts65Dn [33–35] and the Ts1Cje models [80], and in mice transgenic for the *DYRK1A* gene, which is one of the MMU16 genes triplicated in the other two models [36]. Many modulations in monoamine pathways observed in the Ts1Cje male mice were not present in the Ts65Dn mice, showing that there is probably a cross modulation of the effects of some MMU16 genes by other MMU16 genes. The expression of monoamine pathways has not been evaluated in murine models overexpressing MMU17 or MMU10 genes orthologous to HSA21 genes, although impaired synaptic plasticity measured by hippocampal LTP in the Ts65Dn male mice [32, 34, 81] can be restored by some MMU17 genes, including *CBS* [36, 38, 82]. Thus, the present study is the first showing that the overexpression of a gene on MMU17 orthologous to the HSA21 *CBS* gene induces different monoamine contents according to brain area, age, and sex.

The present results reinforce the importance of the murine chromosome 17 genes orthologous to HSA21 genes, especially *CBS*, in relation to the role of H_2S neuromodulator as shown by a recent study [67]. The present results should be considered in conjunction with reports of differences with age and sex in both mouse models and people with Down syndrome. These findings demonstrate a critical need to design both preclinical trials in mouse and clinical trials in people with trisomy 21 to account for both sex and age especially for their responses to any putative drug related to monoamine neurotransmitter disorders in childhood [83, 84], aging [85], and especially trisomy 21.

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Author Contributions DJ, LJ, and RC designed the study; BLC, NDK, and RC performed the HPLC experiments; DF, LJ, RC, and SB performed the other experiments; LJ, NDK, and RC analyzed the data; LJ and RC wrote the paper. JN, LS, and MC corrected the manuscript.

Compliance with Ethical Standards

This study was approved by the Animal Ethical Committee of University Paris Diderot (CEEA-40, approval number CEB-001-2011).

Conflict of Interest The authors declare that they have no conflict of interest.

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