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Cerebral 5-HT_{2A} receptor binding, but not mGluR2, is increased in tryptophan hydroxylase 2 decrease-of-function mice

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Abstract

Transgenic mice with knock-in (KI) of a tryptophan hydroxylase 2 (Tph2) R439H mutation, analogous to the Tph2 R441H single-nucleotide polymorphism originally identified in a late life depression cohort, have markedly reduced levels of 5-hydroxytryptamine (5-HT). These Tph2KI mice are therefore interesting as a putative translational model of low endogenous 5-HT function that allows for assessment of adaptive changes in different anatomical regions. Here, we determined 5-HT_{2A} receptor binding in several brain regions using *in vitro* receptor autoradiography and two different radioligands. When using the 5-HT_{2A} receptor selective antagonist radioligand ^{3}H-MDL100907, we found higher binding in the prefrontal cortex (10%, P=0.009), the striatum (26%, *P*=0.005), and the substantia nigra (21%, P=0.027). The increase was confirmed in the same regions with the 5-$HT_{2A/C}$ receptor agonist, ^{3}H-CIMBI-36 (2-(4-Bromo-2,5-dimethoxyphenyl)-*N*-(2-methoxybenzyl)ethanamine). 5-HT_{2A} receptors establish heteromeric functional receptor complexes with metabotropic glutamate 2 receptors (mGluR2), but binding levels of the mGluR2/3 ligand ^{3}H-LY341495 were unaltered in brain areas with increased 5-HT_{2A} receptor levels. These data show that in distinct anatomical regions, 5-HT_{2A} receptor binding sites are up-regulated in 5-HT deficient mice, and this increase is not associated with changes in mGluR2 binding.

Introduction

Increased 5-hydroxytryptamine 2A (5-HT_{2A}) receptor levels in depression patients and in suicide victims have been reported postmortem [2, 25, 32] and vulnerability factors of depression are positively correlated with frontolimbic 5-HT_{2A} receptor binding potential *in vivo* [12]. Conversely, atypical antidepressants such as nefazodone and mianserin are potent 5-HT_{2A} receptor antagonists [6], although such compounds also target other receptors and transporters.

One mechanism through which cerebral 5-HT_{2A} receptors levels increase in depression could be an autoregulatory response to sustained low 5-HT levels. A moderate 5-HT depletion results in an increase and 5-HT2A receptor levels [5, 16] and chronic treatment

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with selective 5-HT reuptake inhibitors leads to a reduction in 5-HT_{2A} receptor binding [15, 22, 23]. This has also been seen in humans as chronic but not acute treatment with 5-HT uptake inhibitors decreases cortical 5-HT_{2A} receptor binding [24, 29]. It is still unclear if lower 5-HT is the only mechanism leading to an autoregulatory increase in 5-HT_{2A} receptor level or other factors play a role.

A rare decrease-of-function mutation in the coding region of the gene for tryptophan hydroxylase 2 (Tph2) leading to an $^{Arg}441^{His}$ substitution was recently discovered in depressed individuals [34]. Subsequently, a mouse model carrying the identical $^{Arg}439^{His}$ substitution was generated by homologous combination [1]. These Tph2 knock-in (Tph2KI) mice have reduced synthesis-, tissue- and extracellular levels of 5-HT and exhibit increased depression- anxiety- and aggression-like behaviours [1, 18]. The Tph2KI mouse represents an excellent naturalistic model for the study of the long-term consequences of reduced 5-HT-neurotransmission, such as regulation of 5-HT_{2A} receptors by ambient 5-HT levels.

More specific aims of these studies were to correlate any consequence of low 5-HT neurotransmission on 5-HT_{2A} receptor binding to specific anatomical locations using two radioligands with different pharmacological activity on the receptor. Semi-quantitative autoradiography using ^{3}H-MDL100907 was used to measured changes in several cortical and subcortical structures. To examine if changes were taking place in high-affinity 5-HT_{2A} receptor binding sites, we also employed the recently discovered 5-HT_{2A} receptor agonist radiotracer Cimbi-36 [9].

Several lines of evidence suggest that the metabotropic glutamate receptor 2 (mGluR2) establish dimers with 5-HT_{2A} receptors [13, 27]. Activation of 5-HT_{2A} receptor-associated mGluR2 modulates 5-HT_{2A} receptor-mediated cell signalling in a fashion lessening the propensity for hallucinogenic effects [13, 26, 27]. The mGluR2 is therefore considered an important potential target for anti-psychotic treatment [10, 11, 14]. The stoichiometry between the 5-HT_{2A} receptor and mGluR2 may therefore be functionally important [13, 27]. The two receptors exist as either homomeric or heteromeric receptors and activation of homomeric rather than heteromeric receptors would supposedly have different functional responses. In that perspective, we also investigated possible changes in mGluR2 binding using the mGluR2/3 ligand ^{3}H-LY341495 [19] to see if increase in 5-HT_{2A} receptor binding is accompanied by increase in mGluR2 binding.

Materials and Methods

Animals and tissue preparation

The generation of the Tph2KI mice has been described previously [1]. Littermate mice were housed 3–5 per cage with food and water available *ad libitum* on a 12-h light-dark cycle at an ambient temperature of 21+/− 2°C. The mice were quickly euthanized by cervical dislocation and the brains rapidly frozen on dry ice. The brains were sectioned on a cryostat in 12 μm coronal sections, thaw-mounted on superfrost plus glass slides (Thermo Scientific, Braunschweig, Germany), dried, and stored at −80°C until use. Glass slides with 4–5 brain sections were collected at seven rostro-caudal levels of the mouse brain containing the

following seven anatomical regions: prefrontal cortex, striatum, hippocampus, hypothalamus, substantia nigra, brainstem, and cerebellum.

5-HT_{2A} receptor autoradiography

^{3}H-MDL100907 (77 Ci/mmol, kindly donated by Dr. Christer Halldin from Karolinska Institute, Stockholm, Sweden) was used for 5-HT_{2A} receptor autoradiography [20]. ^{3}H-CIMBI-36 (2-(4-Bromo-2,5-dimethoxyphenyl)-*N*-(2-methoxybenzyl)ethanamine) is a 5-HT_{2A} receptor agonist [9], and the labelled compound (53 Ci/mmol) was also kindly provided by Dr. Christer Halldin from Karolinska Institute, Stockholm, Sweden.

The tissue sections were thawed for one hour and pre-incubated at room temperature in 50 mM Tris-HCl, pH 7.4 for 15 min. Incubation was performed in the same buffer containing 1.25 nM ^{3}H-MDL100907 for 60 min, and non-specific binding (NSB) was assessed in adjacent sections in the presence of 10 μM ketanserin tartrate (Tocris Biosciences, Bristol, UK). Sections were washed 2 × 5 min in ice-cold 50 mM Tris-HCl followed by a 20 sec dip in ice-cold dH_2O, and dried on a heating plate (60°C) for 10 min. Finally, sections were fixed in paraformaldehyde vapour overnight at 4°C, and dried for 3 hours in a desiccator at room temperature. The processed slides were together with ^{3}H-microscales exposed to a tritium-sensitive BAS TR2040 phosphor imaging plate (Science Imaging Scandinavia AB, Nacka, Sweden) for 7 days at 4°C. The imaging plate was scanned on a BAS-2500 bioimaging analyser (Fujifilm Europe GmbH, Düsseldorf, Germany).

The buffer used for pre-incubation and incubation with 2 nM ^{3}H-CIMBI-36 was 50 mM Tris-HCl containing 1% BSA and 4 mM $CaCl_2$, pH 7.4. The incubation time used in this experiment was 120 min and the sections washed for 2 × 10 min in the incubation buffer and processed as above.

mGluR2/3 autoradiography

^{3}H-LY341495 (40 Ci/mmol, American Radiolabeled Chemicals, Saint Louis, MO) was used for mGluR2/3 autoradiography [19, 21]. The general procedure was as described for 5-HT_{2A} receptor autoradiography [20] with the following changes: Pre-incubation for 30 min was performed in 50 mM Tris-HCl, pH 7.4. Then the sections were incubated in 2 nM ^{3}H-LY341495 in the same buffer as used for pre-incubation for 90 min. Non-specific binding was determined in the presence of 10 μM glutamate (Sigma-Aldrich, Denmark). Sections were washed for 2 × 30 sec in ice-cold 50 mM Tris-HCl, followed by a30 sec dip in ice-cold dH_2O.

Image analysis of autoradiograms and statistical analysis

Autoradiograms were analysed with Quantity One (version 4.6.8; BioRad, Hercules, CA). Image optical density was determined in the areas of interest in at least three neighbour sections from each animal and background subtracted from each of these measures. The densities were converted to activity density in nCi/mg tissue equivalent using the linear range of ^{3}H-microscales and then converted to radioligand binding in fmol/mg tissue equivalent using the specific activity of the ligands. The specific receptor binding was

determined by subtracting non-specific binding from the total radioactivity in each animal, and the brain regional binding were analysed with Student's *t*-test.

Results

5-HT_{2A} receptors are up-regulated in PFC, striatum and substantia nigra

Receptor autoradiography using the ligand ^{3}H-MDL100907 revealed the characteristic distribution of 5-HT_{2A} receptor binding sites throughout the mouse brain. Binding was completely blocked by co-incubation with ketanserin (Fig. 1). High densities were observed presumably in layer III–IV throughout the neocortex, in the striatum, and in the interpeduncular nucleus. In the striatum, the labeling was heterogeneously distributed and appeared to be most dense in rostral and medial portions (Fig. 1C). Moderate 5-HT_{2A} receptor levels were observed in the prefrontal cortex, frontal cortex, hippocampus, hypothalamus, parietal cortex. Low binding was observed in the substantia nigra, the brainstem, and the cerebellum (Table 1). Overall, higher levels of binding were detected in most of the regions in the Tph2KI mice though the magnitude was different between the brain areas (Table 1). A 10% increase in receptor binding was found in prefrontal cortex ($P < 0.01$) while the binding in the striatum ($P < 0.005$) and substantia nigra ($P < 0.05$) was increased with more than 20% compared to wildtype. Moderate increase was seen in all other regions, though one important exception was the hippocampus where a slight non-significant reduction in binding levels was observed.

The 5-HT_{2A} receptor agonist and radioligand ^{3}H-CIMBI-36 was used to assess if the observed changes in 5-HT_{2A} receptor binding involved high-affinity binding sites. Analysis of the receptor autoradiograms revealed overall the same distribution of binding as described for ^{3}H-MDL100907 (Fig. 2A). However, due to 5-HT_{2C} receptor binding [4], labeling of the choroid plexus was present using ^{3}H-CIMBI-36 (Fig. 2A). Binding of ^{3}H-CIMBI-36 was also significantly higher in the prefrontal cortex, striatum and hypothalamus in the Tph2KI mice (Fig. 2B). The remaining regions also contained a higher level of binding for ^{3}H-CIMBI-36 in the Tph2KI mice, although not statistically significant (Fig. 2B).

mGluR2/3 binding is unaltered

The ratio between 5-HT_{2A} receptors and mGluR2 seems to be important for signalling mediated through the 5-HT_{2A} receptor. In the same animals as used for 5-HT_{2A} receptor binding, receptor autoradiography using ^{3}H-LY341495 was conducted. Quantification of labelling was performed in the two areas where the most significant 5-HT_{2A} receptor binding changes were observed, the prefrontal cortex and the striatum. However, mGluR2/3 binding as determined by ^{3}H-LY341495 autoradiography was not different between wild type and Tph2KI mice (Fig. 3B).

Discussion

We here report that 5-HT deficiency due to impaired endogenous 5-HT synthesis in the Tph2KI transgene mice results in significantly increased 5-HT_{2A} receptor binding levels in the prefrontal cortex, striatum, and the substantia nigra. The increase in binding was

detected using both ^{3}H-MDL100907 and ^{3}H-CIMBI-36; an antagonist and agonist radioligand with different efficacies on the 5-HT$_{2A}$ receptor, respecitively [4, 9, 20].

Similar effects on 5-HT$_{2A}$ receptors have been reported in other models where 5-HT levels are reduced by mechanical or neurotoxic lesions of the dorsal raphe, or by depletion of the 5-HT precursor tryptophan [8, 16, 31]. Furthermore, increase in 5-HT$_{2A}$ receptor binding has been reported but only after chronic and not acute tryptophan depletion [5]. Chronic tryptophan depletion increases ^{3}H-ketanserin binding in the cortex, but not hippocampus [5]. Binding studies and detection of 5-HT$_{2A}$ receptor mRNA levels revealed increases in B_{max} and mRNA suggesting that the number of 5-HT$_{2A}$ receptors is increased rather than binding properties [31]. The increase in binding also reflects a higher sensitivity to the 5-HT$_{2A/C}$ receptor agonist DOI in the Tph2KI mice as the number of head twitches in response to DOI is increased in the Tph2KI mice compared to wildtype [18].

Assuming that all 5-HT neurons produce less 5-HT and the reduction in neurotransmission may occur in all target areas, it is somewhat surprising that the 5-HT$_{2A}$ receptor is only changed in some brain areas and not in other. The region-specific regulations of 5-HT$_{2A}$ receptors could be explained by the synaptic localization of the 5-HT$_{2A}$ receptor. A common characteristic of the brain areas with significant alterations in 5-HT$_{2A}$ receptor binding is they receive a high density of 5-HT nerve fibres [17]. In the cortex, the receptor is primary located on glutamatergic pyramidal neurons, while in the hippocampus the majority of receptors are located on GABAergic interneurons [33]. Decreased 5-HT$_{2A}$ receptor binding has been found in the hippocampus of depressed individuals, while binding is increased in other regions [7, 30]. This is interesting, because the same pattern of receptor changes seen in the Tph2KI mice.

Another possibility is the synaptic distribution of 5-HT$_{2A}$ receptors change in response to 5-HT levels. In the prefrontal cortex, 5-HT$_{2A}$ receptors modulate the release of dopamine and glutamate [3, 28] and it is likely that 5-HT$_{2A}$ receptor antagonistic antidepressants may partly exert their therapeutic action by reducing the activity of pyramidal neurons in mPFC [6].

The differences in 5-HT$_{2A}$R binding between transgenic and wildtype mice were seen both with the agonist and the antagonist, suggesting that the regulatory effect of the lower 5-HT levels also affected high-affinity binding sites.

In contrast to 5-HT$_{2A}$ receptor binding, the mGluR2/3 binding was not changed in the Tph2KI mice. The two receptors form heteromeric complexes [13] and activation of mGluR2 inhibits 5-HT$_{2A}$ receptor signalling [26]. This effect is considered to be an important mechanism through which mGluR2 agonist exert anxiolytic and anti-psychotic effects [10]. This suggests that it is only as monomers that 5-HT$_{2A}$ receptors upregulate in response to lower 5-HT levels.

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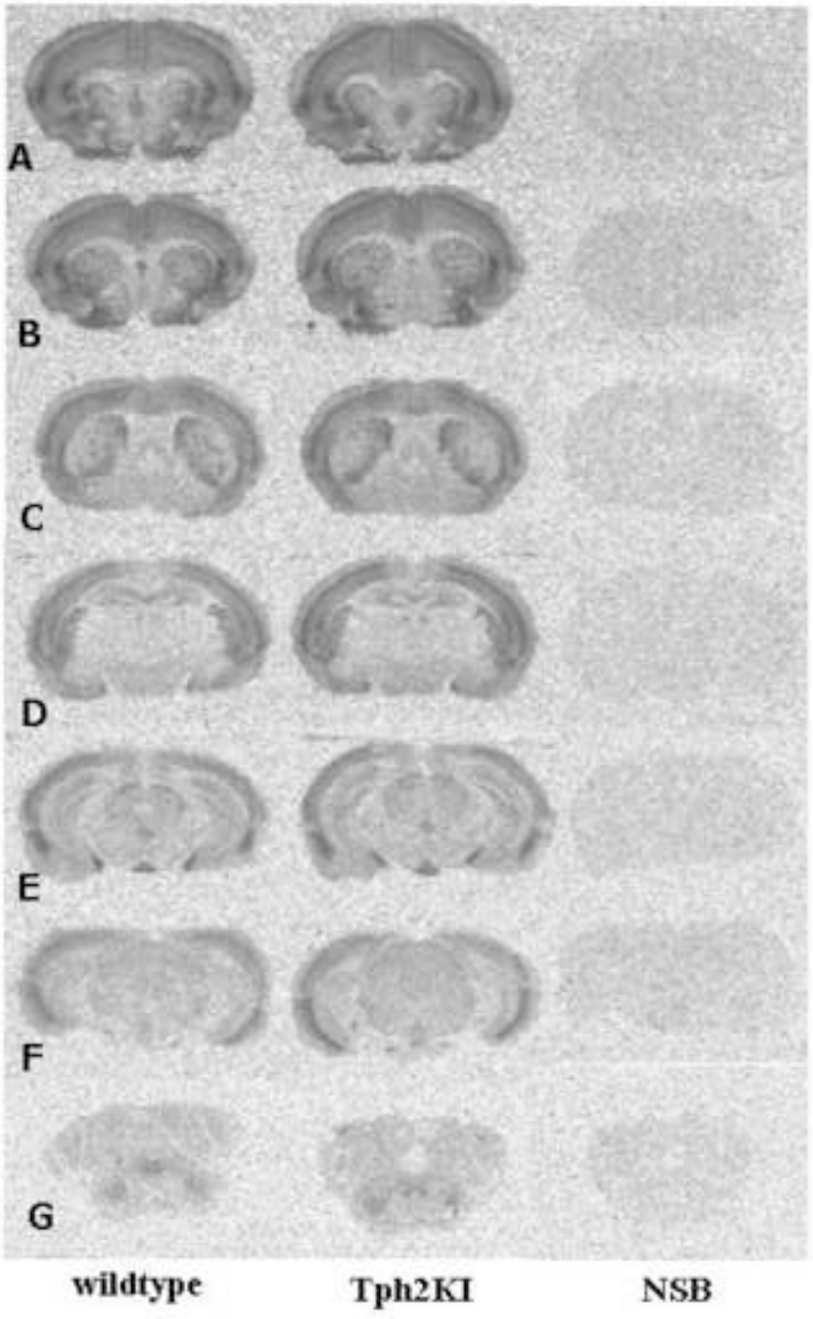

Figure 1.

Binding of ^{3}H-MDL100907 in wild type and Tph2KI mice. Representative autoradiograms throughout the mouse brain of 5-HT_{2A} receptor binding. Specific ^{3}H-MDL100907 binding (1.25 nM) and non-specific binding in the presence of 10 μM ketanserin. n=6–10.

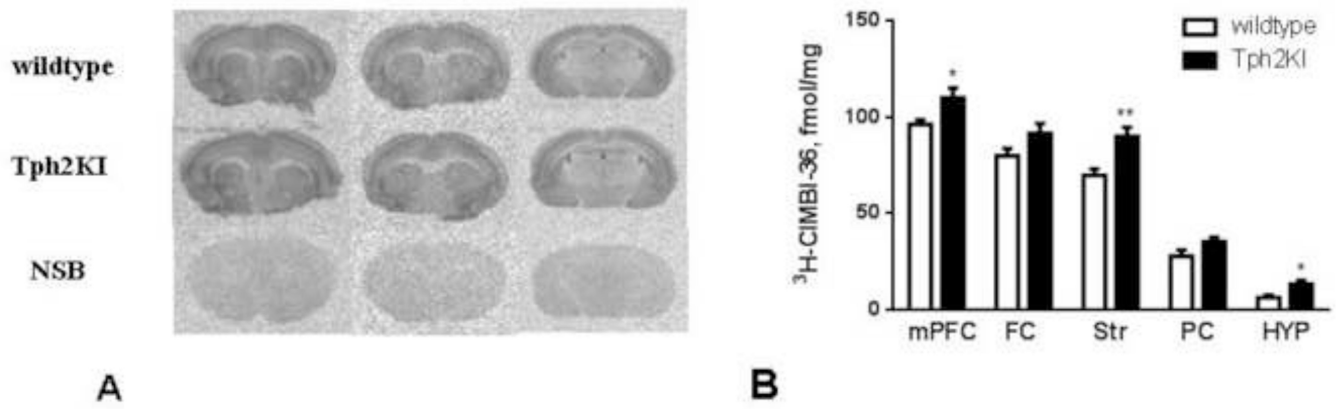

Figure 2.

Receptor binding in wild-type and Tph2KI mice with the radioligand ^{3}H-CIMBI-36 (2 nM). Examples of receptor autoradiograms using ^{3}H-CIMBI-36 demonstrate binding in the cortex and the striatum. Non-specific binding was assessed in the presence of 10 μM ketanserin (A). The quantitative data are presented as means ± S.E.M (B). Student's unpaired *t*-test. n=7–10.

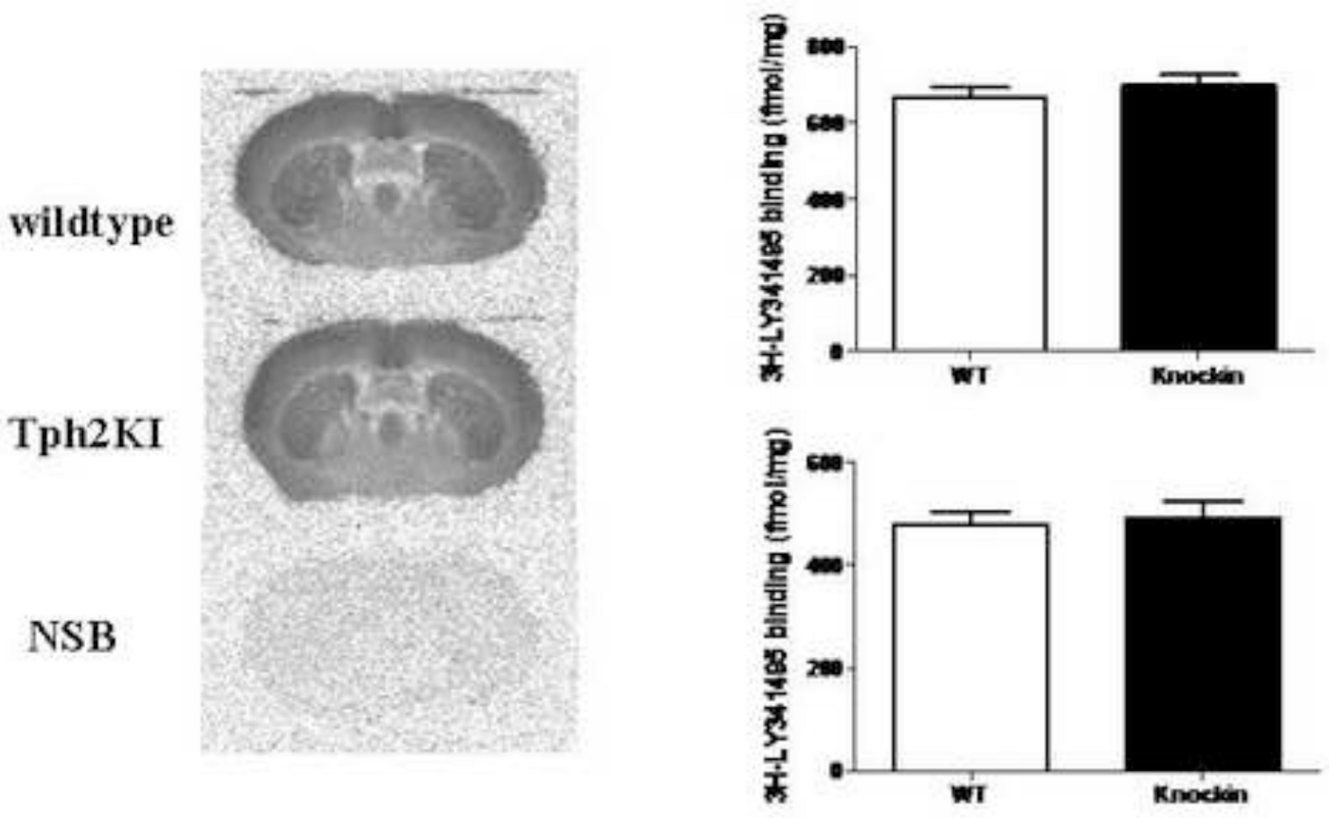

Figure 3.

Receptor binding using ^{3}H-LY341495 binding (2 nM) in the striatum. The quantitative data are presented as means ± S.E.M. Student's unpaired *t*-test. n=8–10.

Table 1

5-HT_{2A} receptor binding in wildtype and tph2KI mice. Densiometric measurements were performed on autoradiograms from sections incubated with ^{3}H-MDL100907 in different brain regions. The data are expressed as binding in areas of interest and the relative change and statistical difference as revealed by Student t-tests are indicated. Significant changes are present in the prefrontal cortex, the striatum, and the substantia nigra.

	Wildtype (fmol/mg)	**n**	**Tph2KI (fmol/mg)**	**n**	**% change**	**P-value**
Prefrontal cortex	48.40 ± 1.16	7	53.24 ± 1.07	8	10.00	0.0088 **
Frontal cortex	39.54 ± 1.34	10	42.38 ± 1.33	8	7.18	0.1271
Striatum	20.54 ± 1.09	10	25.86 ± 1.21	8	25.90	0.0048 **
Parietal cortex	30.67 ± 1.38	6	33.45 ± 1.38	8	9.06	0.1898
Hippocampus	15.09 ± 0.75	7	14.25 ± 0.69	7	−5.57	0.4202
Hypothalamus	12.55 ± 0.49	6	15.06 ± 1.10	7	20.00	0.0734
Substantia nigra	6.24 ± 0.34	8	7.52 ± 0.39	8	20.51	0.0271*
Brainstem	7.48 ± 0.34	9	8.30 ± 0.40	8	10.96	0.1323
Cerebellum	2.66 ± 0.32	9	2.88 ± 0.30	7	8.27	0.6296