Densities of Serotonergic Projections as Revealed by In Situ Synthesised Labelled α -Methyl-Serotonin: an Autoradiographic Evaluation

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Abstract

An estimate of serotonergic innervation density and regional serotonin (5-HT) concentration was performed from the distribution of in situ produced labelled α -methyl-serotonin. Rats were injected with (^3H) labelled α -methyl-L-tryptophan and the tracer distribution was measured using the autoradiographic method 14 days following the injection. In a separate experiment, the total brain concentration of 5-HT in the rat brain was found to be 2.4 ± 0.2 nmol/g. Based on this, and the assumption that the specific activity of in situ produced α -methyl-serotonin is the same as that of the injected tracer, it was possible to estimate the regional concentrations of 5-HT and the relative concentration of regional serotonergic innervations. It was found, and reported for the first time here, that the highest concentration of serotonergic innervation is present in the solitary nucleus. Regionally measured 5-HT concentrations accord well with previously reported concentrations of 5-HT.

Keywords

Tracer; α -Methyl-L-tryptophan; Serotonergic innervations; Rat brain

Introduction

It has been suggested that disturbances to 5-HT neurotransmission play an important role in a wide range of neuropsychiatric disorders. 5-HT has been implicated in many brain functions, from those related to pleasure and intake of food, to depression, Parkinson's and Alzheimer's diseases (See, for example, [1, 30]). Changes in 5-HT neurotransmission have also been implicated in the mediation of the clinical efficacy of most antidepressant medications.

5-HT synthesis from the essential amino acid, L-tryptophan (L-Trp), is a two-step enzymatic process. Tryptophan is hydroxylated through the action of tryptophan hydroxylase (TPH) and molecular oxygen, with tetrahydrobiopterin as a coenzyme. TPH is considered to be the rate-limiting enzyme in 5-HT synthesis [12]; in the brain, it is found exclusively in 5-HT neurons [14]. The product of the Trp hydroxylation reaction, 5-hydroxy-L-tryptophan, undergoes decarboxylation through the action of aromatic amino acid decarboxylase (AAAD), which is also found in catecholaminergic neurons and catalyzes the decarboxylation of 3,4-dihydroxy-L-phenylalanine. 5-HT is metabolized to 5-HIAA (5-hydroxyindoleacetic acid) by MAO (monoamine oxidase) and aldehyde dehydrogenase and is removed from the CNS (central nervous system) into the CSF (cerebrospinal fluid), as well as the blood, through a probenecid-sensitive mechanism [5].

Serotonergic projections originate from the cell bodies found in the brain stem mainly in the dorsal (DR) and median (MR) raphe nuclei [2, 19, 21, 25, 26, 28]. These serotonergic projections are relatively diffuse, meaning that they are found more or less in all brain regions. Because of these somewhat diffused projections, the serotonergic system has been involved and/or implicated in many brain related disorders [1, 30]. In addition to 5-HT projections from the DR and MR in these structures, neurons containing noradrenaline, GABA, neurotensin, dopamine and substance P have been identified [27]. For a better understanding of the functional significance of these projections, more information is required about the densities and organization of these projections. There have been many studies of brain regional innervation using different methods to visualize the presence of serotonin (5-HT) containing neurons. The pioneering work on the localization of 5-HT containing cells and their projections was done by Björklund et al. [3] using the Falck-Hillarp histochemical fluorescence method. This work has been followed by many others using a variety of ex vivo methods [2, 19, 21, 25, 26, 28]. In those studies, the densities of the projections were qualitative. However, some attempts to quantify serotonergic projections were made by Descaries et al. [7] and Oleskevich and Descaries [18], through the use of tritium labelled 5-HT. These studies identified 5-HT neurons as those taking up labelled 5-HT, however the quantification of projections this way may be influenced by a local ratio between endogenous 5-HT and exogenous labelled 5-HT, which is not homogeneous (not fully equilibrated). In addition, there could be some uptake of labelled 5-HT into the noradrenergic and/or dopaminergic neurons because the transporters of different monoamines can be promiscuous [29].

The tracer labelled α -MTrp is transported into the brain similarly as endogenous Trp, and the permeability surface area for the α -MTrp correlates well with endogenous Trp [11].

Further, the conversion of Trp into 5-HT correlates with the trapping of labelled α -MTrp in the brain. The tracer, labelled α -MTrp, is a substrate for TPH [15]. The hydroxylation product is acted upon by AAAD, and the α -M5-HT formed is trapped for long periods in the brain tissue [15], as α -M5-HT is not a substrate for MAO [22] and does not readily cross cell membranes. This results in the accumulation of labelled α -M5-HT in the brain tissue. α -MTrp is not incorporated into the proteins [8, 22] and it is not a substrate for tryptophan pyrrolase [24]. Moreover, it does not undergo (in normal conditions), with significance, any peripheral or central metabolism. From the experimental data collected so far there is only one metabolic pathway in the normal brain responsible for trapping the labelled compound. The tracer levels of α -M5-HT produced in situ are stored in the 5-HT neuronal pool and can be released from the brain tissue slices upon depolarization with potassium [6]. This accumulation in the tissue serotonergic pool and at tracer levels (in situ produced tracer for 5-HT) permits the localization and determination of the distribution of the natural congener (5-HT) without any pharmacological intervention. In this mode, the procedure is able to evaluate 5-HT distribution related to the in vivo activity of TPH [6], because it has been shown that there is an excellent co-localization between the labelled tracer (both α -MTrp and α -M5-HT) [8, 22], TPH and endogenous 5-HT [6]. On the assumption that the vesicles are packed with the same number of molecules of the neurotransmitter and because of the constant ratio between the tracer and endogenous neurotransmitter, one can estimate the number of neuronal projections in a particular brain region. As we did not measure the actual densities in any of the brain regions, the projection density is calculated relative to the density in the frontal cortex.

Materials and Methods

In the present study, six male Sprague–Dawley (SD) rats, obtained from a local supplier, weighing between 200 and 250 g were used. The rats were habituated in the Animal Facility for at least 72 h before being used in the experiments. The rats were housed with a 12 h light–dark cycle and had free access to food and water. However, the night before the tracer injection, the rats were deprived of food but given water ad libitum. Fasting was necessary to obtain stable concentrations of the plasma amino acids [10, 16]. Under light halothane (1.0–2.0%) anaesthesia, a plastic catheter was inserted into the femoral vein (for the tracer injection). The rats were attached to lead bricks with a masking tape put over their casts to prevent them from moving. The rats were then allowed to awaken before the tracer injection. One mCi of (3H)-labelled α -methyl-L-tryptophan (Specific activity = 10 Ci/mmol; custom synthesis preparation by Amersham (GE Healthcare)), dissolved in saline, was injected into each of the rats. This means that approximately 0.1 micromol of labelled α -MTrp was injected into each rat, which is not expected to interfere with the steady state metabolism of tryptophan in the brain or other parts of the body. The tracer was injected intravenously in 1 ml of saline over 2 min, using a constant infusion with a Harvard Apparatus injection pump. The tracer has an initial plasma half life of distribution of approximately 1 min with a terminal half-life of about 50 min [9]. The rats were killed by guillotine 14 days following the tracer injection. The brains were then removed, frozen in isopentane (cooled to -20°C) and stored at -80°C until they were cut in a cryotome (-20°C) into 20 μm thick coronal sections. The brain sections were mounted on glass slides and exposed to Kodak tritium

sensitive film along with ^3H -polymer standards for up to 6 months to obtain the autoradiograms.

All animal use procedures were in strict accordance with the Canadian Council on Animal Care guidelines, and were approved by the Animal Care Committee of McGill University.

The images were digitized using a microcomputer-based image analysis system (MCID; Imaging Research Inc., St. Catharines, Ont., Canada) consisting of a video camera, a frame grabber and a personal computer. Tissue radioactivity concentration (nCi/g) was calculated from optical density using a calibration curve made by plotting the optical density of ^3H -standards as a function of their tissue equivalent concentration. In most cases, a third-order polynomial was utilized as a calibration curve for conversion of the optical density into the tissue tracer concentration (nCi/g). The outlining regions of interest (ROI) were placed on each structure. The tissue concentration of the tracer was measured separately in 38 brain regions. An average of six readings in each area in three consecutive sections and in each rat was obtained. The different brain areas were identified by the aid of the rat brain atlas [20]. Sections of the DR and Amy were separately quantified, as shown in Fig. 1A, B, respectively. After the analysis of the readings for different parts of the DR and the benzo lateral Amy (the other parts have a rather low innervation), we decided to present the data as one result, as no additional information was obtained when the structures were presented separately.

Because labelled α -MTrp is converted into ^3H -labelled α -methyl-serotonin (α -M5-HT) in the rat brain after 14 days [8, 15], albeit at a relatively slow rate, only labelled α -M5-HT remains. α -M5-HT is not a substrate for MAO and as it is a charged molecule at its physiological pH, it takes a long time for it to leave the brain [17]. An estimated half-life for the conversion of α -MTrp into α -M5-HT in the rat brain is approximately 180 min [8, 15]. From this, one can calculate that after 14 days, all of the tracer would be in the α -M5-HT form. This was confirmed by the HPLC in one of the rats; there was no measurable amount of labelled α -MTrp present in the brain after 14 days (Diksic et al. unpublished observation). In vivo synthesized α -M5-HT is stored in vesicles and it can be released through depolarization with potassium [6]. A released fraction of labelled α -M5-HT is approximately the same as a released fraction of neuronal 5-HT [6]. α -M5-HT has been identified as a non-selective agonist for several 5-HT receptors [13]. As such, when in vivo, it is most likely 100% recycled because it is not a substrate for MAO [22] and it does not pass through the blood brain barrier.

In a separate set of experiments, the total 5-HT brain concentration was measured by HPLC in five rats. The rats were between 200 and 245 g. Following euthanasia, the brain was extracted within 2 min and frozen (-80°C) until processed. During the processing stage, the brains were thawed and homogenized in four volumes of 25% tri-chloroacetic acid. Following centrifugation at $10,000\times g$, the 5-HT concentration was measured in supernatant using HPLC [8]. Three measurements for each rat were performed and the mean values averaged. The mean concentration of 5-HT in the whole brain was found to be 2.4 ± 0.2 nmol/g-brain. Because α -MTrp was used at tracer levels and as it was shown, both at the tracer [6, 8] and pharmacological [15] levels, that it is metabolically converted into S- α -M5-

HT (the S-form has been identified by us; Diksic and S. Rajagopal, unpublished), it was possible to calculate the amounts of 5-HT present in each structure and obtain information on the density of the innervations. The fact that in situ produced labelled α -M5-HT was found to be at tracer levels and was a tracer for endogenous 5-HT, it was possible to obtain, from the regionally measured concentration of labelled α -M5-HT through autoradiography, a very accurate regional concentration of 5-HT throughout the rat brain. This is supported by the fact that it is reasonable to assume that 5-HT and α -M5-HT are in full equilibrium in different brain regions.

Based on these concentration levels and brain tissue radioactivity following α -MTrp injection, the amounts of 5-HT in different brain regions can be calculated, as the ratio between labelled α -M5-HT and endogenous 5-HT should be the same in all brain regions. This follows from the fact that labelled α -methyl-L-tryptophan and its metabolite, α -methyl serotonin, were at tracer levels. Using the 5-HT concentration measurements in the brain regions, absolute amounts of 5-HT in different brain regions could be estimated from the amounts of α -M5-HT present (as measured in this study) in each brain region.

It follows, from these assumptions, that regional tracer concentration is directly related to the density of serotonergic innervations. It is not possible to know, from this experiment, if the concentration of this metabolite is the same in each terminal. However, there is a strong possibility that after 14 days, the concentrations in each of the terminals are approximately the same, which would permit a calculation of the number of terminals in different regions.

Results and Discussion

Serotonergic innervations expressed relatively to the frontal cortex were found to be significantly different between the different brain regions (Table 1; presented as the mean $\pm$ standard error of the mean (SEM)). The greatest density of serotonergic innervations was found in the Sol (solitary), followed by the ventromedial hypothalamus, and somewhat lower densities were found in the LC, Amy, Hyp-DM, and AN. The densities of serotonergic innervations in different cortical regions were very similar, but not uniform. Similarly, the innervations of the caudate-putamen are also not uniform; the medial part of this structure has a density that is more than two times greater than the density in the lateral part. The mean innervation of the caudate-putamen is almost two times greater than that of the cortical regions, but more than ten times lower than in the Sol or Hyp_VM (Table 1). The innervation of the ventral hippocampus was more than two times greater than the innervation of the dorsal part, or approximately double that of the CA3 layer measured in the ventral region of the hippocampus. The serotonergic innervation densities in the hypothalamus were found to be rather heterogenous with densities in the VM almost five times greater than in the LH. The Hyp_PaV has densities that are three times lower than the Hyp_VM and approximately 50% greater than in the LH. Comparisons between the relative innervations (Table 1, column 2) and the concentrations of TPH in different brain regions [4] suggest a much larger variability of the innervations (Table 1; column 2) and 5-HT concentrations (Table 1; column 4) than concentrations of TPH [4]. For example, the concentrations of TPH are approximately the same in different parts of the Amy [4], but the innervation measurements clearly indicate a rather large heterogeneity, with the largest concentration present in the

benso lateral Amy (Table 1; Fig. 2D). This heterogeneity suggests a non uniform packing of 5-HT in different brain regions.

The brain regions containing dopaminergic cells (*e.g.*, SN, VTA) have densities of serotonergic innervations several fold greater than those in the brain cortical regions. There was also a larger difference in the innervations of the SNc and SNr, the former having almost double the density of innervations than the latter. Very dense serotonergic innervations were observed in the LC, the noradrenergic cell body region. These innervations were more than double those found in the dopaminergic cell body regions (*e.g.*, SN, VTA).

A relatively large amount of tracer was accumulated in the serotonergic cell body structures, including the brain stem raphe nuclei. In the raphe nuclei, the tracer accumulated in the cell bodies (representing the serotonin pool), dendrites, and the terminals projecting from other raphe nuclei (raphe-raphe connections). The greatest accumulation was observed in the DR, followed by the MR and Rpo. The smallest accumulation was observed in the RM, about one quarter of which was in the DR. In the DR, we observed rather clear distinctions of different parts of this structure, and noted that at varying levels, its different parts are clearly delineated (Fig. 2E). Similarly, innervations to different parts of the hypothalamus have been observed. The dentate gyrus and different layers of the hippocampus (CA1, CA2 and CA3) do not have a very large differentiation of serotonergic innervations (Fig. 2D). The relative densities of the innervations are in general accordance. It is difficult to compare to the qualitative measurements reviewed by Steinbusch [26], as our results are more quantitative and, as such, show a larger structure to structure variability. A particularly large difference is found in the Sol, which is identified to have “low density” in the review by Steinbusch [26], while, in the present work, it has the largest serotonergic innervation (Table 1; column 2). There is also a discrepancy in the concentration of TPH [4] and 5-HT measurements in the Rpa, Rob and Rpo presented here (Table 1; column 4). This discrepancy could be related to the difficulties in sampling three small brain structures for TPH measurements.

Differences between 5-HT concentrations calculated from the tracer amounts in individual brain regions (Table 1, column 4) and those reported by Saavedra and Palkovits [23] suggest there is a very large difference in 5-HT storage in different terminals. As depicted in Fig. 3, the differences between regional 5-HT concentrations calculated from the innervation densities and specific activity of α -M5-HT in different brain regions (it is assumed that the specific activity in different brain regions is the same as that in the frontal cortex) varied greatly. The most significant difference was found in the solitary track (Sol), followed by the Hyp_DM and amygdala. It is possible that the primary reason for these discrepancies, particularly those in the LC and Sol, is the rather small size of these brain regions, which could affect the dissection samples for HPLC measurements. These differences could also indicate a variance in 5-HT packing in the terminals in different brain regions, as reported by Descarries et al. [7].

The 5-HT concentration distinctions measured in different raphe nuclei and those calculated from the measurements with labelled α -MTrp (reported here) were also variable, with the largest difference being in the Rpa and the smallest in the RM (Fig. 3). This suggests that 5-

HT packing in different nuclei do not have the same densities, which could be related to varying amounts of 5-HT, per vesicle, a different number of vesicles per cell, and/or a different number of 5-HT cells per unit of brain volume. It has been previously reported that there is a difference in the number of axonal varicosities in different brain structures, as measured by electron microscopic images [7]. It is not surprising that the measurements in the pineal body with this tracer and those performed by tissue sampling and HPLC do not accord (Table 1), as the pineal body does not have a blood brain barrier and the tracer would be lost over the 14 days.

In conclusion, it can be stated that based on the findings of the present study, the greatest serotonergic innervations are present in the Sol. This observation is reported for the first time here. The data also suggest, as previously reported [7], that vesicular packing is not the same in all of the projection areas.

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Abbreviations

5-HT	5-Hydroxytryptamine
5-HIAA	5-hydroxyindolacetic acid
AAAD	Aromatic amino acid decarboxylase
L-Trp	Tryptophan
TPH	Tryptophan hydroxylase
α-MTrp	A-methyl-L-tryptophan
α-M5-HT	A-methyl-5-hydroxytryptamine
MAO	Monoamine oxidase
HPLC	High performance liquid chromatography
GABA	γ -Aminobutyric acid L-tryptophan
SD	Sprague–Dawley
AMY	Amygdala
AN	Accumbens
CA3	CA3 of Ammon's Horn
Cl	Clastrum
CPm	Caudate putamen medial

CPI	Caudate putamen lateral
CxA	Auditory cortex
CxCin	Cingulate cortex
Cxf	Frontal cortex
CxEnt	Entorhinal cortex
CxP	Parietal cortex
CxS	Sensory cortex
CxV	Visual cortex
DR	Dorsal raphe
GP	Globus pallidus
HiD	Dorsal hippocampus
HiV	Ventral hippocampus
Hyp_DM	Hypothalamus dorso-medial
Hyp_LH	Lateral hypothalamus
Hyp_PaV	Paraventricular hypothalamic nucleus
Hyp_VM	Ventromedial hypothalamic nucleus
IC	Inferior colliculus
LC	Locus coeruleus
LG	Lateral geniculate
MBF	Medial forebrain
MG	Medial geniculate
MR	Median raphe
P	Pineal body
SC	Superior colliculus
SNe	Substantia nigra compact
SNr	Substantia nigra reticular
SO	Superior olive
Sol	Solitary tract
RM	Raphe magnus

Rpa	Raphe pallidus nucleus
Rob	Raphe obscure nucleus
Rpo	Raphe pontine
ThD	Dorsal thalamus
ThV	Ventral thalamus
VTA	Ventral tegmental area

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Fig. 1.

A schematic presentation of the regions read as DR-d (dorsal raphe-dorsal part), DR-v (ventral part), and DR-lat (lateral part) part A, and parts of amygdala part B. These were adapted from the cross-sections provided in Paxinos and Watson (1997; with permission)

Fig. 2. Autoradiograms qualitatively showing serotonergic innervation at different brain cross sections. The levels shown in inserts A (Bregma -13.2 mm), B (Bregma -8.0 mm), C (Bregma 1.0 mm) and D (Bregma -3.6 mm) are identified by approximate distances from the Bregma. Insert E depicts the different parts of the DR, as schematically shown in Fig. 1, insert A

Fig. 3.

Percentage differences between 5-HT concentrations measured with α -MTrp as a tracer and those measured by HPLC in discrete brain regions [23]. The abbreviations of the brain structures are provided in the list of abbreviations

Table 1

Tracer concentrations (nCi/g) in individual brain regions as measured with labelled α -MTrp 14 days following the tracer injection and the calculated concentration of 5-HT (nmol/g-brain) in different regions using the measured value in the whole brain, as described in the Methods section

	Ratio relative to Frontal Cortex Mean $\pm$ SEM	Tracer Density (nCi/g) Mean $\pm$ SEM	Calculated 5-HT Concentration (nmol/g-brain) Mean $\pm$ SEM	5-HT (nmol/g-brain); from ref. [23]
AMY	13.3 $\pm$ 3.8	119.5 $\pm$ 13.8	12.99 $\pm$ 1.50	10.46
AN	7.9 $\pm$ 1.5	71.4 $\pm$ 5.4	7.76 $\pm$ 0.59	8.34
CI	2.2 $\pm$ 0.1	19.4 $\pm$ 0.4	2.11 $\pm$ 0.04	NM
CPI	1.0 $\pm$ 0.1	9.4 $\pm$ 0.4	1.02 $\pm$ 0.05	9.93
CPm	2.4 $\pm$ 0.3	21.3 $\pm$ 1.3	2.31 $\pm$ 0.14	10.78
CxA	1.2 $\pm$ 0.2	10.4 $\pm$ 0.6	1.13 $\pm$ 0.06	NM
CxCin	0.9 $\pm$ 0.1	8.1 $\pm$ 0.4	0.88 $\pm$ 0.04	5.25
CxEnt	1.5 $\pm$ 0.2	13.2 $\pm$ 0.7	1.43 $\pm$ 0.08	4.94
CxF	1.0 $\pm$ 0.0	9.0 $\pm$ 0.4	0.98 $\pm$ 0.04	2.08
CxP	1.0 $\pm$ 0.1	8.9 $\pm$ 0.5	0.97 $\pm$ 0.06	3.01
CxS	1.0 $\pm$ 0.1	8.8 $\pm$ 0.4	0.96 $\pm$ 0.05	NM
CxV	1.1 $\pm$ 0.1	9.7 $\pm$ 0.5	1.06 $\pm$ 0.06	NM
GP	2.2 $\pm$ 0.2	19.8 $\pm$ 0.7	2.16 $\pm$ 0.07	10.78
CA3	3.4 $\pm$ 0.3	30.6 $\pm$ 2.5	3.33 $\pm$ 0.6	NM
HiD	1.1 $\pm$ 0.1	10.3 $\pm$ 0.5	1.12 $\pm$ 0.06	6.36
HiV	2.8 $\pm$ 0.3	25.1 $\pm$ 1.0	2.73 $\pm$ 0.11	NM
Hyp_DM	13.4 $\pm$ 3.7	119.9 $\pm$ 13.5	13.04 $\pm$ 1.47	7.72
Hyp_LH	5.7 $\pm$ 0.3	51.1 $\pm$ 1.2	5.56 $\pm$ 0.13	NM
Hyp_PaV	18.0 $\pm$ 4.3	161.6 $\pm$ 15.6	17.57 $\pm$ 1.69	7.66
Hyp_VM	4.2 $\pm$ 0.4	38.1 $\pm$ 1.6	4.15 $\pm$ 0.18	4.82
IC	1.1 $\pm$ 0.2	9.9 $\pm$ 0.6	1.07 $\pm$ 0.07	1.53
LC	15.6 $\pm$ 0.9	139.7 $\pm$ 3.1	15.19 $\pm$ 0.34	5.49
LG	3.3 $\pm$ 0.5	29.2 $\pm$ 1.7	3.18 $\pm$ 0.19	5.67
MBF	6.2 $\pm$ 0.4	55.8 $\pm$ 1.5	6.06 $\pm$ 0.16	16.63
MG	1.5 $\pm$ 0.2	13.1 $\pm$ 0.8	1.42 $\pm$ 0.09	3.23
P	28.9 $\pm$ 3.0	261 $\pm$ 16	28.3 $\pm$ 1.8	438
DR	18.5 $\pm$ 0.0	166.0 $\pm$ 0.1	18.06 $\pm$ 0.01	15.09
MR	12.9 $\pm$ 0.9	115.7 $\pm$ 3.2	12.58 $\pm$ 0.35	12.94
RM	3.7 $\pm$ 0.5	33.4 $\pm$ 2.0	3.63 $\pm$ 0.22	8.74
Rob	5.9 $\pm$ 0.9	53.3 $\pm$ 3.4	5.79 $\pm$ 0.37	7.04
Rpa	9.1 $\pm$ 1.6	81.8 $\pm$ 5.9	8.90 $\pm$ 0.64	5.56
Rpo	5.5 $\pm$ 0.8	49.4 $\pm$ 3.1	5.38 $\pm$ 0.34	4.60
SC	2.6 $\pm$ 0.2	23.5 $\pm$ 0.9	2.56 $\pm$ 0.10	6.64
SNC	5.0 $\pm$ 0.6	45.3 $\pm$ 2.1	4.93 $\pm$ 0.23	11.01
SNr	2.8 $\pm$ 0.3	25.1 $\pm$ 1.0	2.73 $\pm$ 0.11	11.92
SO	3.2 $\pm$ 0.5	28.5 $\pm$ 1.8	3.10 $\pm$ 0.20	2.50

	Ratio relative to Frontal Cortex Mean $\pm$ SEM	Tracer Density (nCi/g) Mean $\pm$ SEM	Calculated 5-HT Concentration (nmol/g-brain) Mean $\pm$ SEM	5-HT (nmol/g-brain); from ref. [23]
Sol	26.9 $\pm$ 4.6	241.2 $\pm$ 16.9	26.23 $\pm$ 1.84	5.50
ThD	1.4 $\pm$ 0.2	12.8 $\pm$ 0.8	1.39 $\pm$ 0.09	5.50
ThV	1.1 $\pm$ 0.1	10.1 $\pm$ 0.5	1.10 $\pm$ 0.06	NM
VTA	7.8 $\pm$ 0.7	70.2 $\pm$ 2.6	7.63 $\pm$ 0.28	12.6

Six rats were used for the measurements. When applicable, the values are provided as mean $\pm$ SEM (standard error of the mean)