5-HT_{2A} receptor antagonist M100907 reduces serotonin synthesis: An autoradiographic study

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Abstract

The effects of the administration of the serotonin (5-HT)_{2A} antagonist, M100907, on 5-HT synthesis rates, were evaluated using the α -[¹⁴C]methyl-L-tryptophan (α -MTrp) autoradiographic method. In the treatment study, M100907 (10 mg/kg) was injected intraperitoneally 30 min before the α -MTrp injection (30 μ Ci over 2 min). A single dose of M100907 caused a significant decrease in the synthesis in the anterior olfactory nucleus, accumbens nucleus, frontal cortex, sensory-motor cortex, cingulate cortex, medial caudate-putamen, dorsal thalamus, substantia nigra, inferior colliculus, raphe magnus nucleus, superior olive, and raphe pallidus nucleus.

These data suggest that the terminal 5-HT_{2A} receptors are involved in the regulation of 5-HT synthesis in the entire brain. Further, 5-HT synthesis is likely regulated by the 5-HT_{2A} antagonistic property of M100907 in the cortices, anterior olfactory nucleus, caudate putamen, and nucleus accumbens.

Keywords

α -[¹⁴C]methyl-L-tryptophan; Serotonin synthesis rate; 5-HT_{2A} receptor antagonist; M100907; Autoradiography

1. Introduction

Serotonin (5-HT) cell bodies contained in the raphe nuclei have both ascending and descending axonal projections, and there are serotonergic projections from these cell bodies throughout the brain. 5-HT influences brain functions through the activation of numerous 5-HT receptor subtypes [4,14,64]. The 5-HT₂ receptor family is comprised of the 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors, which readily associate with the Gq family of G-proteins and upon activation, stimulate phospholipase C to increase phosphoinositide metabolism and cause a transient rise in intracellular free calcium [44,65,60]. The 5-HT₂ receptors have been reported to play a role in a remarkable range of central nervous system disorders, including anxiety, depression, migraine, obsessive compulsive disorder, excess impulsivity, anorexia

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nervosa, schizophrenia [1,12,5,21]. They also influence sleep and eating patterns [1,12,5,21]. Further, it has been shown that the administration of drugs with an affinity for the 5-HT_{2A} receptors (e.g., 5-HT_{2A} antagonists) can act synergistically with selective serotonin reuptake inhibitors (SSRIs) [43], by offering potential therapeutic advances in the treatment of some neuropsychiatric disorders [45].

5-HT₂ type binding sites were initially defined by their low affinity for [³H]5-HT and a high affinity for [³H]spiperone [40,58]. The density of 5-HT₂ receptors in the rat brain is highest in the claustrum, olfactory tubercle and layer IV of the cerebral cortex [56,67]. The 5-HT_{2A} receptors were also present in the anterior olfactory nucleus, piriform cortex, layers I and V of the neocortex, caudate putamen, nucleus accumbens, dentate gyrus, mammillary bodies, thalamus, hippocampus, brainstem, medulla, cerebellum and spinal cord [56,67]. 5-HT_{2A} receptor density was elevated in the olfactory bulbectomized (OBX) rats, a rat model of depression, and, antidepressant treatment down-regulated the density of the 5-HT_{2A} receptors in the OBX rats [67]. Given that the 5-HT_{2A} receptor shows distinct regional distributions, it is possible that it modulates specific physiological functions (e.g., “Executive Function”; [27]), as well as 5-HT synthesis.

The putative antipsychotic drug, *R*(+)-alpha-(2,3-dimethoxy-phenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidine-methanol (M-100907), is a highly selective 5-HT_{2A} receptor antagonist with a significantly greater affinity (>500-fold) for the 5-HT_{2A} receptor over the D2 receptor [53,22], which may underlie its ability to achieve a high level of receptor occupancy in the absence of extrapyramidal-like symptoms in humans [3]. M100907 has been shown to elicit a positive response in a large number of preclinical paradigms designed to detect antipsychotic activity [38]. Several of these rodent paradigms indicate that M100907 can reverse the behavioral effects of an acute hyperdopaminergic state that has been induced by amphetamines [70], potentially through modification of its stimulated release of dopamine in the medial prefrontal cortex [57] and nucleus accumbens shell [42]. This stimulated release of dopamine has likely influence on serotonergic neurotransmission through neurotransmitter interaction [28]. As it has been stated by Nelson [50]: “the most important steps in the neurosecretion cycle are the synthesis of specific neurotransmitters in the presynaptic cell, accumulation of the transmitter into synaptic vesicles or secretory granules, measured secretion of the transmitter into synaptic cleft and reuptake of the neurotransmitter or its derivative”. This clearly emphasizes that synthesis is a very important factor in neurotransmission and its control and its modulation needs to be investigated in more detail, as well as its relation to changes in behavior, before a good understanding of any antidepressant action can be obtained.

The autoradiographic method using labeled α-[¹⁴C]methyl-L-tryptophan (α-[¹⁴C] MTrp) for the measurement of regional 5-HT synthesis rates in the rat brain [49,37] relies on the unidirectional uptake (trapping) of α-MTrp, which is transported into the brain and has been shown to be converted, in part, to α-methyl-5-HT [23]. The trapping constant can be converted into 5-HT synthesis rates by division with a lumped constant (LC), which represents a conversion constant [23], followed by multiplication with the plasma free tryptophan (Trp; [49]).

Because data has implicated the involvement of 5-HT receptors in several brain disorders and given that an alteration in the functionality of 5-HT_{2A} receptors has been reported in several affective disorders, it was felt to be important to evaluate a drug that acts on that receptor. It is also important to investigate whether 5-HT_{2A} receptors play a role in the modulation of 5-HT synthesis, as the modulation of regional 5-HT synthesis appears to be part of antidepressant action [37,23,10,30,46]. This question was studied by investigating the influence of a selective 5-HT_{2A} antagonist, M100907. The effects of M100907 on 5-HT synthesis in the rat brain using the autoradiographic α -[¹⁴C]MTrp method were investigated.

2. Materials and methods

2.1. Animals

Sprague–Dawley male rats (Charles River) weighing (mean $\pm$ SEM) 213 $\pm$ 6 g (control rats) and 229 $\pm$ 5 g (treatment group) were housed in the animal facility (room temperature of 22 $\pm$ 2 °C with a 12-h day-night cycle) for at least 3 days before the administration of the drugs. The animals were fasted overnight to stabilize the plasma concentration of tryptophan (Trp), as well as the other amino acids, but water was given *ad libitum* before the α -[¹⁴C]MTrp autoradiographic experiments. The stabilization of other amino acids is needed because several essential amino acids share the same transport system with Trp at the blood brain barrier [13,66,71]. To avoid any possible influence of the circadian rhythm on the measurements, the tracer was injected between 11:00 AM and 1:00 PM, and all of the rats were sacrificed between 1:00 PM and 3:00 PM. The body weight of each rat was recorded before the initial treatment of the drug. All surgical procedures and experiments were performed with the approval of the Animal Care Committee of the Montreal Neurological Institute of McGill University, and were done according to the procedures of the Canadian Council on Animal Care.

2.2. Drug

M100907 was synthesized according to the published procedure [76] and identification was performed using the melting point determination, ¹H NMR, and MS. The compound was dissolved in saline (0.9% NaCl). The control rats received saline injections. A dose of 10 mg/kg of M100907 or saline at a volume of 2 mL/kg was administered (with 12 rats in each group). Both the drug and saline were administered intraperitoneally 30 min before the injection of α -[¹⁴C]MTrp. α -[¹⁴C]MTrp was synthesized using the previously described procedure [48] and had a specific activity of 55 mCi/mmol.

2.3. Experimental procedure

The femoral artery and vein were cannulated with plastic catheters under light halothane (1.0–2.0%) anesthesia. The posterior limbs of the rats were fixed using a loose-fitting plaster cast, and the rats were allowed to awaken. The body temperatures of the rats were kept at approximately 37 °C with a heated lamp. In the acute treatment study, a dose of 10 mg/kg M100907 in 2 mL/kg of saline was injected intraperitoneally 2 h following the surgical treatment. The same volume of saline was injected into the control rats in the same manner. Thirty minutes after the drug injection, 30 μ Ci of α -[¹⁴C]MTrp in 1 mL of saline was

injected through a catheter into the femoral vein over 2 min by an injection pump (Harvard Apparatus, Model 55-2226).

Arterial blood samples (40 μ L each) were taken at progressively larger intervals, starting from the beginning of the tracer injection to the decapitation of the rats (taken at: 0.5, 1, 1.5, 2, 3, 5, 10, 20, 30, 45, 50, 55, 60 min for the 60 min experiments, and at: 0.5, 1, 1.5, 2, 3, 5, 10, 30, 60, 90, 120, 140, 145, 150 min for the 150 min experiments). The total volume of the blood taken was about 0.56 mL, and the blood was always replaced by saline. The blood samples were centrifuged for 3 min at 9,300 g, and 20 μ L of plasma was taken for liquid scintillation counting to measure the plasma radioactivity needed for an arterial input function. Physiological parameters of arterial samples (PO_2 , PCO_2 , pH, and hematocrit) were measured at least twice in each experiment. Five additional blood samples were taken to measure the plasma concentrations of total and free Trp, using the method described below.

The rats were guillotined 60 or 150 min following the tracer injection, as required by the experimental protocol [49]. The brains were removed, frozen in cold 2-methylbutane, and sliced into 30 μ m thickness in a cryostat at -20°C . The brain slices were mounted on glass slides and exposed to X-ray films along with ^{14}C -polymer standards (American Radiolabel Co., St. Louis, MO, USA; calibrated to 30 μ m thickness of the brain tissue) for 3 weeks to obtain the autoradiograms.

2.4. The measurement of plasma Trp concentration

Five plasma samples (total of about 0.4 mL; always replaced by saline) were taken at different times to determine the total and free (non-albumin-bound) Trp concentrations in the plasma. Forty μ L of plasma was deproteinized with 20 μ L of 20% trichloroacetic acid and used to measure the total Trp concentration in the plasma. After the sample was mixed with a vortex-mixer and centrifuged, 40 μ L of supernatant was stored in a freezer (-84°C) until it was analyzed for total Trp. An additional 40 μ L of plasma was filtered through a Biomax-10 filter (10,000 MW cutoff, Millipore Co., Bedford, MA, USA) spinning at $9300 \times g$ for 10 min, and was also stored in a freezer (-84°C) until it was analyzed for free Trp. The total and free Trp concentrations were measured using HPLC (high performance liquid chromatography) with fluorescence detection [71].

2.5. Measurement of the α -MTrp trapping and calculation of 5-HT synthesis

A set of representative autoradiograms illustrating 5-HT synthesis in the rat brain as determined by the α -MTrp method have been presented in many previous publications [49,37,24,52,75,30,31] and their addition would not provide any new information, because the logarithmic scale between the tracer concentration (represented by 5-HT synthesis) and optical density. All that can be seen from these images is the brain non-homogenous distribution. The resultant images on the X-ray film were analyzed using a microcomputer-based image analyzing system (MCID/M4-Image Analysis System, Imaging Research Inc., Canada) and tissue equivalent calibrated standards as detailed in previous publications [49,37,23,30]. The optical densities were converted into tissue radioactivity concentration ($n\text{Ci/g}$). The tissue concentrations of the tracer were measured separately in 39 brain

structures. Averages of six readings (bilaterally) in each brain structure in three consecutive sections was obtained. In this investigation the dorsal raphe was not separated into the dorsal, ventral or lateral parts. Instead, the tracer concentration of the entire structure was measured in all of the slices and the average value was used in the calculation of 5-HT synthesis. The tissue radioactivity concentrations were converted into volume of distribution (DV^* ; mL/g) by dividing tissue concentrations [$C^*(T)$; nCi/g] by the plasma tracer concentration [$C^*(T)$; nCi/mL] at the end of the experiment. There was a significant linear relationship between DV and Θ (min) [$\Theta = \int_0^T C^*(t)dt / C^*(T)$] with a slope K (mL/g/min; trapping constant for α -[^{14}C]MTrp as previously described [49,24] in all of the structures investigated. Note that the rats killed at different clock times would have different exposure time Θ [min] making a better definition of the straight line. The rate of 5-HT synthesis R ; pmol/g/min is calculated by converting K (slopes obtained from fits of DV as function of Θ) to K^T ($K^T = K/LC$) and then multiplying the calculated K^T by the plasma free (non-protein-bound) Trp (C_p ; pmol/mL); $R = C_p K/LC = C_p K^T$. The use of plasma free Trp in this calculation is based upon reports indicating that this plasma fraction is in dynamic equilibrium with brain Trp [13,66,71]. The LC was measured in vivo and found to be 0.42 ± 0.07 [78], uniform throughout the rat brain. In the present experiments, all of the linear relationships between Θ [min] and DV^* [mL/g] had a significant ($p < 0.05$; F -statistics) and positive slope [49].

2.6. Statistical analysis

The statistical evaluation of the physiological group data was performed by an analysis of variance (ANOVA). The regional synthesis in the control and treatment groups was analyzed by ANOVA. After finding a significant interaction between brain regions (note that the pineal body was excluded because it is outside the brain) and treatment groups, the region specific ANOVA was evaluated. The region specific ANOVA was post hoc corrected by the Benjamini–Hochberg false discovery rate multiple tests correction [7] before significance was assigned. In all cases the statistical significance was defined as $p < 0.05$.

3. Results

There were no significant differences in the physiological parameters (e.g., pH, PaCO_2 , PaO_2) and hematocrit between the control and treated groups ($F(1,21) = 0.014$; $p > 0.9$). The mean weight in the treated (229 ± 5 g) rats was significantly different ($F(1,21) = 4.6$; $p < 0.05$) from the control (213 ± 6 g) rats. There was no significant difference in the concentration of plasma free or total Trp between the treatment and respective control groups ($F(1,21) = 0.51$; $p > 0.4$). In the control group (mean $\pm$ SEM), plasma-free Trp was 11.4 ± 1.1 nmol/mL and in the M100907 group, it was 12.8 ± 1.4 nmol/mL. The total Trp concentrations were 92 ± 8 nmol/mL and 101 ± 10 nmol/mL in the control and treated groups, respectively.

The 5-HT synthesis rates measured in 38 brain structures and pineal body of the rat brain following administration of M100907 or saline are provided in Table 1. The synthesis rates in the M100907 treated rats were, overall (group effect), significantly lower than in the respective control group ($F(1,21) = 4.6$; $p < 0.05$). The region-group interaction was highly

significant ($F(1,21) = 18.7$; $p < 10^{-10}$). There was no region in which drug produce a significant increase in 5-HT synthesis following treatment. On the other hand, the synthesis rates in almost all of the projection areas that were investigated were decreased when compared to the control group. However, a significant reduction was found in 12 out of 38 brain regions. Additionally, 7 brain regions had $p < 0.05$ but lost significance after the false discovery rate correction (Table 1). The largest decrease was in the inferior colliculus (71%), frontal cortex (42%), sensory motor cortex (33%), cingulate cortex (35%), caudate putamen medial (33%), dorsal thalamus (32%), substantia nigra compacta (30%), raphe magnus nucleus (26%), superior olive (66%), and raphe pallidum nucleus (35%). Evaluation of the correlation between synthesis reduction (present results) and density of 5-HT_{2A} receptors, taken from [67], suggest a trend towards correlation between these variables which is not significant ($r = -0.417$; $p = 0.14$; number of pairs; $N = 11$).

4. Discussion

In this study, we quantitatively evaluated the effects of the administration of a specific 5-HT_{2A} receptor antagonist, M100907, using the α -MTrp autoradiographic method on regional 5-HT synthesis rates in the rat brain. The synthesis rates in the M100907 treatment group was, overall, significantly lower than in the control group and 5-HT synthesis rates were reduced on average by $24 \pm 15\%$ (Table 1). Significant decreases of 5-HT synthesis rates were seen in the cortices, anterior olfactory nucleus, accumbens nucleus, caudate putamen (Table 1), where there is an enriched density of 5-HT_{2A} receptors in the rat brain [29]. This suggests a relationship between density of 5-HT_{2A} receptors and reduction of serotonin synthesis (the trend exist showing higher reduction for regions with higher densities of receptors). Because 5-HT_{2A} receptors are postsynaptic influence on 5-HT synthesis is most likely through postsynaptic loops and/or modulated by other neurotransmitters (e.g., dopamine; many 5-HT_{2A} receptors are found on dopaminergic neurons; [47]). The action through 5-HT_{2A} receptors is probably also related to 5-HT dopamine interaction because blockade of 5-HT_{2A} receptors on dopaminergic neurons could result in higher release of dopamine which could influence 5-HT synthesis [35]. The tryptophan hydroxylase (TPH) is “mostly” expressed in the cell bodies found in raphe nuclei (e.g., [36]), but there is also substantial evidence that TPH is present in the serotonergic terminals [62,18,59], and that the synthesis of 5-HT is occurring in those terminals (e.g., [49,33,11,51]).

The data from the present study suggest that the 5-HT synthesis rates in the midbrain raphe nuclei and medulla raphe nuclei are not equally affected by acute treatment with M100907 (Table 1). The synthesis is decreased in the medulla raphe nuclei and there is no effect in the midbrain raphe nuclei as a whole. It was previously reported that 5-HT_{2A} receptors are found in the brain stem and medulla [14,25,32,79], however, these sites were not found in the midbrain [6,26]. The present data also suggest a different influence on 5-HT synthesis in these parts of the brain through treatment.

The 5-HT synthesis rate in the claustrum and visual cortex were reduced. However, there were no significant differences (Table 1). The 5-HT_{2A} sites that were found to be enriched

were the claustrum, a region that is connected to the visual cortex, some components of the limbic system, particularly the olfactory nuclei, and parts of the basal ganglia [34].

The study of the brain distribution of 5-HT_{2A} receptors showed a large densities in the layer V of the neocortices, entorhinal cortex, cingulate cortex, anterior olfactory nucleus, nucleus accumbens, striatum, claustrum, and globus pallidus [67,55,41,19]. The density in the dorsal raphe nucleus is very low and possibly not existent [19]. These distributions may have significant functional implications for the pharmacological treatment of some neuropsychiatric disorders.

5-HT_{2A} receptors are located postsynaptically in brain regions known to be involved in learning, such as the neocortex, which includes the limbic cortex, hippocampus, thalamus and basal ganglia [29]. As can be seen in Table 1, while not throughout the brain, the 5-HT_{2A} antagonist, M100907, did produce a detectable decrease of 5-HT synthesis rates in the neocortex, some limbic areas, and the thalamus (Table 1).

As 5-HT_{1A} and 5-HT_{2A} receptors have been particularly implicated in the regulation of mood, a functional imbalance may exist between the 5-HT_{1A} and 5-HT_{2A} receptors in brains of patients with mood disorders [15,8]. Long-term treatment with SSRIs produces a desensitization of hypothalamic 5-HT_{1A} receptors, both in humans and rats [9,39,61] and alters the function of 5-HT_{2A} receptors [16,73,20]. Recent studies suggest that the activation of 5-HT_{2A} receptors induces a desensitization of the 5-HT_{1A} receptors [77,81]. It is possible that SSRIs exert their therapeutic activity, at least in part, by restoring the balance of sensitivity between the 5-HT_{1A} and 5-HT_{2A} receptors. For example, combining a 5-HT_{2A} receptor antagonist, such as the atypical antipsychotic drug olanzapine, with fluoxetine, improves the therapeutic efficacy in treatment-resistant depression [68,72]. Additionally, the mechanism of the antipsychotic effects of atypical antipsychotic drugs may involve an intricate interaction between the 5-HT_{1A} and 5-HT_{2A} receptors [35,46]. We have pointed to a 5-HT receptor desensitization following chronic treatment with the 5-HT_{1A} agonist, buspirone [52], the 5-HT_{1B} agonist, CP93-129 [31], the 5-HT_{1A/1B/1D/2C} agonist, TFMPP and the 5-HT_{1A/1B} agonist, CGS12066B [75].

Several studies have reported elevated 5-HT_{2A} receptor density and/or 5-HT_{2A} receptor-mediated responses in depressed patients and the normalization of this abnormality through antidepressant treatment [2,54,63]. Additionally, the reduction of the 5-HT_{2A} receptors by the administration of antisense oligonucleotide produced an antidepressant-like effect in the forced swim test in mice [69]. Recently, several studies have suggested that the addition of drugs with significant 5-HT_{2A} antagonist properties, such as atypical antipsychotic drugs or antidepressants (e.g., mianserin and mirtazapine), to SSRIs enhance therapeutic responses in major depression [43,17]. Together, these findings (present and previous) indicate that the desensitization of 5-HT_{2A} receptor function may be an essential part of antidepressant drug action.

We have shown that elevated 5-HT synthesis rates in an animal model of depression, olfactory bulbectomized (OBX) rats, were normalized following chronic SSRI treatment [30]. It was thought that normalized 5-HT synthesis rates resulted in the desensitization of

the 5-HT_{1A} receptor [30]. Additionally, it has been suggested that the desensitization of the 5-HT_{2A} receptor function occurs through chronic treatment with either fluvoxamine or paroxetine. This could be the case as a result of prolonged exposure to elevated levels of 5-HT [81,80], which suggests that 5-HT_{2A} receptors interact directly with 5-HT_{1A} receptors in neurons of the hypothalamic periventricular nucleus, or that hypothalamic neurons expressing 5-HT_{2A} receptors interact with other hypothalamic neurons that express 5-HT_{1A} receptors. This observation suggests that a direct intracellular interaction occurs between these receptor signaling systems. From this, it can be considered that chronic treatment of a 5-HT_{2A} antagonist using OBX rats is needed.

The M100907 compound investigated here did not have any influence on either the plasma free or total-Trp.

In conclusion, we demonstrated that in the acute treatment study, the 5-HT synthesis rates of the cortices, some limbic regions, and the raphe nuclei in the medulla oblongata were decreased following the administration of the selective 5-HT_{2A} receptor antagonist, M100907. This suggests a different coupling of 5-HT_{2A} receptors with the second messengers than those of 5-HT_{1A}, because at the latter receptors, agonists reduced [52,75], and antagonists [74] elevated, regional 5-HT synthesis.

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Abbreviations

5-HT	5-hydroxytryptamine
α-MTrp	α-methyl-L-tryptophan
SSRI	selective serotonin reuptake inhibitor
M100907	R(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidine-methanol
OBX	olfactory bulbectomized rats
DV*	volume of distribution
LC	lumped constant
HPLC	high performance liquid chromatography
ANOVA	analysis of variance
TPH	tryptophan hydroxylase

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Table 1

The effect of saline (control) and M100907 (10 mg/kg, i.p.) on 5-HT synthesis rates (pmol/g/min) in the rat brain.^a

Brain region	Mean $\pm$ SEM control (N = 11)	Mean $\pm$ SEM treatment (N = 12)	F(1,21); <i>p</i> ^c	% Reduction ^d
Anterior olfactory nucleus	65.4 $\pm$ 3.5	48.8 $\pm$ 3.3	12.0; 0.0023	25
Accumbens nucleus	58.2 $\pm$ 3.2	42.3 $\pm$ 3.1	12.67; 0.0019	27
Frontal cortex	48.3 $\pm$ 3.6	27.8 $\pm$ 3.5	16.7; 0.0005	42
Sensory-motor cortex	34.1 $\pm$ 2.8	22.9 $\pm$ 2.7	8.06; 0.0098	33
Cingulate cortex	44.7 $\pm$ 3.4	29.3 $\pm$ 3.3	10.8; 0.0036	35
Caudate putamen (lateral)	47.7 $\pm$ 2.9 ^b	37.7 $\pm$ 2.8	6.22; 0.02	21
Caudate putamen (medial)	56.1 $\pm$ 3.5	37.8 $\pm$ 3.3	14.6; 0.00099	33
Caudate putamen	45.4 $\pm$ 2.9	40.4 $\pm$ 2.8	NS	11
Clastrum	51.4 $\pm$ 3.4	45.0 $\pm$ 3.3	NS	13
Globus pallidus	46.7 $\pm$ 3.1	37.9 $\pm$ 3.0	NS	19
Medial forebrain bundle	40.9 $\pm$ 3.2	32.1 $\pm$ 3.1	NS	22
Parietal cortex	37.3 $\pm$ 2.9 ^b	27.3 $\pm$ 2.8	6.05; 0.02	27
Hippocampus (dorsal)	53.9 $\pm$ 3.0	46.7 $\pm$ 2.9	NS	13
Thalamus (dorsal)	39.9 $\pm$ 3.1	27.1 $\pm$ 2.9	9.07; 0.0066	32
Thalamus (ventral)	40.4 $\pm$ 2.9 ^b	30.3 $\pm$ 2.8	6.26; 0.02	25
Amygdala	48.8 $\pm$ 2.9	44.7 $\pm$ 2.8	NS	8
Hypothalamus	49.3 $\pm$ 3.2	40.2 $\pm$ 3.1	NS	19
Lateral geniculate body	41.8 $\pm$ 3.0 ^b	32.0 $\pm$ 2.8	5.73; 0.03	24
CA3	59.9 $\pm$ 3.2	56.5 $\pm$ 3.1	NS	6
subiculum	42.0 $\pm$ 3.0	36.9 $\pm$ 2.9	NS	12
Auditory cortex	38.0 $\pm$ 3.0	31.8 $\pm$ 2.8	NS	16
Hippocampus (ventral)	57.6 $\pm$ 3.5	53.9 $\pm$ 3.3	NS	7
Medial geniculate body	43.9 $\pm$ 3.2 ^b	32.8 $\pm$ 3.1	5.73; 0.03	25
Substantia nigra compacta (SNc)	41.9 $\pm$ 3.1	29.4 $\pm$ 3.0	8.38; 0.0087	30
Substantia nigra reticulata (SNr)	34.3 $\pm$ 3.0	27.5 $\pm$ 2.9	NS	20
Ventral tegmental area	48.1 $\pm$ 3.3 ^b	36.8 $\pm$ 3.2	6.05; 0.02	24
Superior colliculi	32.0 $\pm$ 3.5	30.9 $\pm$ 3.3	NS	3
Medial geniculate body dorsal	41.5 $\pm$ 3.3	32.8 $\pm$ 3.2	NS	21
Visual cortex	37.8 $\pm$ 3.3	30.8 $\pm$ 3.2	NS	19
Entorhinal cortex	51.5 $\pm$ 3.6	44.7 $\pm$ 3.5	NS	13
Dorsal raphe nucleus	178.7 $\pm$ 6.2	177.8 $\pm$ 5.9	NS	0.5
Median raphe nucleus	117.7 $\pm$ 6.3	106.0 $\pm$ 6.1	NS	10
Pontine raphe nucleus	63.1 $\pm$ 4.1 ^b	51.1 $\pm$ 3.9	4.41; 0.048	19
Inferior colliculi	22.1 $\pm$ 3.3	6.4 $\pm$ 3.2	11.6; 0.0027	71
Magnus raphe nucleus	54.3 $\pm$ 3.6	40.5 $\pm$ 3.5	7.6; 0.012	26
Superior olive	33.7 $\pm$ 4.1	11.5 $\pm$ 3.9	15.1; 0.00085	66
Obliquus raphe nucleus	47.1 $\pm$ 4.8	35.3 $\pm$ 4.6	NS	25

Brain region	Mean $\pm$ SEM control ($N = 11$)	Mean $\pm$ SEM treatment ($N = 12$)	$F(1,21); p^c$	% Reduction ^d
Pallidus raphe nucleus	54.4 $\pm$ 4.4	35.1 $\pm$ 4.3	9.78; 0.005	35
Pineal body	876 $\pm$ 48	816 $\pm$ 37	NS	7

^aThe values are presented as mean $\pm$ SD (standard deviation). The SD was calculated from the variance of the linear regression [49].

^bIdentifies the brain region which lost significance after False Discovery post hoc correction.

^cBelow are F values with associated degrees of freedom in parenthesis for all brain regions with $p < 0.05$. NS stands for not significant; which means that comparing values in the control and treatment groups had $p > 0.05$.

^dThe percentages of reduction were calculated relative to the synthesis in the control group.