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The opposite effect of a 5-HT$_{1B}$ receptor agonist on 5-HT synthesis, as well as its resistant counterpart, in an animal model of depression

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Abstract

Flinders Sensitive Line (FSL) rat is as an animal model of depression with altered parameters of the serotonergic (5-HT) system function (5-HT synthesis rates, tissue concentrations, release, receptor density and affinity), as well as an altered sensitivity of these parameters to different 5-HT based antidepressants. The effects of acute and chronic treatments with the 5-HT$_{1B}$ agonist, CP-94253 on 5-HT synthesis, in the FSL rats and the Flinders Resistant Line (FRL) controls were measured using α-[^{14}C]methyl-L-tryptophan (α-MTrp) autoradiography. CP-94253 (5 mg/kg), or an adequate volume of saline, was injected i.p. as a single dose in the acute experiment or delivered via the subcutaneously implanted osmotic minipump (5 mg/kg/day for 14 days) in the chronic experiment. The acute treatment with CP-94253 significantly decreased the 5-HT synthesis in both the FRL and FSL rats, with a more widespread effect in the FRL rats. Chronic treatment with CP-94253 significantly decreased 5-HT synthesis in the FRL rats, while 5-HT synthesis in the FSL rats was significantly increased throughout the brain. In both the acute and chronic experiment, the FRL rats had higher brain 5-HT synthesis rates, relative to the FSL rats.

The shift in the direction of the treatment effect from acute to chronic, using the 5-HT$_{1B}$ agonist, CP-94253, on 5-HT synthesis in the FSL model of depression, with an opposite effect on the control FRL rats, suggests the differential adaptation of the 5-HT system in the FSL and FRL rats to chronic stimulation of 5-HT$_{1B}$ receptors.

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Conflict of interest
None.

Keywords

Flinders Sensitive Line; Flinders Resistant Line; Depression; 5-HT_{1B} receptor; α-MTrp; Autoradiography; Serotonin synthesis rate

1. Introduction

The Flinders Sensitive Line (FSL) of rats was selectively bred from the Sprague-Dawley (SPD) strain for their supersensitivity to the hypothermic action of the anticholinesterase agent, diisopropyl fluorophosphate (DFP) [33,34]. The Flinders Resistant Line (FRL) of rats was selectively bred from the same background strain as the FSL rats (SPD), for their decreased sensitivity to the hypothermic effects of DFP, and represent the most used control strain in research on FSL rats (reviewed by Overstreet [33]). The concomitant findings of the cholinergic supersensitivity in a subset of depressed humans led to the postulation of the cholinergic theory of depression and motivated the research on FSL rats as a possible model of depression [17].

FSL rats exhibit face validity (phenomenological similarity with the symptoms of depression in humans) by showing lower activity in the open field [7] and forced swim tests [12,24] (resembling psychomotor retardation in a subset of depressed humans), decreased reward-sensitivity under stressful conditions [42] (resembling anhedonia B decreased ability to experience pleasure) and decreased social interaction [35] (resembling social anxiety, frequently co-morbid with depression in humans) (reviewed by Overstreet et al. [36] and Yadid et al. [54]). Research at the neurochemical level suggests that the behavioural phenotype of FSL rats results from alterations in multiple neurotransmitter systems, including the cholinergic [32], serotonergic (5-HT) [15,18], glutamatergic [24], and GABA-ergic [13,40], as well as at the level of the second messenger signalling systems [10]. The FSL model of depression has a very good predictive validity, i.e., selective sensitivity of neurochemical and behavioural features of the model to treatment with clinically effective antidepressants, including those acting on the 5-HT system, but not other psychotropic drugs, such as stimulants [36]. Alterations of the 5-HT system in the FSL rats include 6–8-fold higher tissue levels of 5-HT in the limbic regions, along with similar levels of extracellular 5-HT [56], lower levels of the vesicular monoamine transporter in dopaminergic terminals in the nucleus accumbens [45], lower 5-HT synthesis [15], lower densities of 5-HT_{1A}, and higher densities of 5-HT_{1B} receptors [30].

5-HT synthesis is an important component of 5-HT transmission, as the quantities of 5-HT available for release directly depend on 5-HT synthesis levels. It has been shown that both types of 5-HT receptors with altered densities in the FSL rats (5-HT_{1A} and 5-HT_{1B}) are involved in the control of 5-HT synthesis [9,50]. The 5-HT_{1B} receptor is localized at the synaptic terminals of the 5-HT neurons, where it serves as an autoreceptor involved in the regulation of 5-HT neuron functioning. It is also localized at the cholinergic, dopaminergic (DA), glutamatergic and GABA-ergic neurons, regulating the neurotransmitter release from these neurons [4]. Stimulation of 5-HT_{1B} receptors decreases the concentration of the cyclic adenosine monophosphate second messenger in the target neurons [4]. The 5-HT_{1B} receptor

has been implicated in the regulation of several physiological functions such as sleep, locomotor activity, sexual behaviour and appetite [4], many of which are altered in FSL rats [36], as well as in a subset of depressed patients (Diagnostic and Statistical Manual of Mental Disorders (DSM-IV)). Specifically, FSL rats have been proposed as a model for psychomotor retardation [42], a state of cognitive and motor slowing frequently found in major depression (DSM-IV) [2]. The aim of the present study is to assess the role of 5-HT_{1B} receptors in the control of 5-HT synthesis in FSL rats, a rat model of depression, relative to the control FRL strain, taking into account the previously established role of 5-HT_{1B} receptors in the control of 5-HT synthesis in normal rats [14], the higher density of 5-HT_{1B} receptors, and the lower baseline 5-HT synthesis in FSL rats, relative to the FRL controls [15,30].

Following the chronic treatment of normal rats with fluoxetine, an indirect agonist of 5-HT_{1B}, the adaptation of 5-HT_{1B} signalling was demonstrated both at the level of mRNA expression [26] and receptor sensitivity [28]. Therefore, an additional aim of the present study was to assess the adaptability of the 5-HT_{1B} receptors in the FSL rats and FRL controls to chronic stimulation. The effect of acute (5 mg/kg i.p.) and chronic (5 mg/kg/day i.p., for 14 days) treatments with the selective and blood–brain barrier penetrating 5-HT_{1B} agonist, 5-propoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-pyrrolo [3,2-b]pyridine hydrochloride (CP-94253), on 5-HT synthesis, in 35 brain regions in the FSL and FRL rats, using α-[^{14}C]methyl-L-tryptophan (α-MTrp) ex vivo autoradiography (reviewed by [8]) was studied. CP-94253 shows a higher affinity for 5-HT_{1B} receptors relative to both the 5-HT_{1D} and 5-HT_{1A} receptors (25- and 40-fold, respectively) [21].

2. Materials and methods

2.1. Animals

FSL and FRL rats (10 weeks of age and weighing 190–240 g at the outset of the treatment) from the in-house colony at the Montreal Neurological Institute were housed two per cage in the animal facility (room temperature of 22 ± 2 °C with a 12-h day–night cycle). The breeding colonies were kindly provided by Dr. David Overstreet (Center for Alcohol Studies, University of North Carolina, Chapel Hill, NC 27599-7178, USA). All surgical procedures and experiments were performed with the approval of the Animal Care Committee of the Montreal Neurological Institute of McGill University, and according to the procedures of the Canadian Council on Animal Care.

2.2. Drug

CP-94253 hydrochloride (Tocris Bioscience, Ellisville, MO, USA) was dissolved in saline (0.9% NaCl). In the acute experiment, an i.p. injection (5 mg/kg) was administered 30 min prior to the infusion of the α-MTrp. The volume of the drug solution injected was ~0.2 mL. The control animals received the same volume of saline. In the chronic experiment, the drug solution was delivered using the osmotic mini-pumps (Alzet model 2ML2; ALZA, Palo Alto, CA, USA), with the release rate of 120 μL/day, for 14 days. The drug concentration in the solution was tailored to the mini-pump release rate and the predicted mean weight of the animal throughout the treatment period, to achieve a daily dose of 5 mg/kg. The dose of

CP-94253, chosen for the acute and chronic experiment (5 mg/kg), was based on that which produced an antidepressant-like response in the forced swim test in mice [49].

3. Experimental

3.1. Minipump implantation

The rats were assigned to either groups receiving 5 mg/kg day of CP-94253 dissolved in 120 μL of saline (Flinders Resistant Line-Chronic-Treatment, FRL-CHR-TR; Flinders Sensitive Line-Chronic-Treatment, FSL-CHR-TR groups) or the same volume of saline alone (Flinders Resistant Line-Chronic-Saline, FRL-CHR-SAL; Flinders Sensitive Line-Chronic-Saline, FSL-CHR-SAL groups). Osmotic mini-pumps were implanted subcutaneously at the dorsum of the rat, under general isoflurane anaesthesia (5% for induction and 2% for maintenance). Minipumps are used to ensure the constant drug delivery rate. The skin over the implanted mini-pump was closed with sutures and treated with povidone-iodide. Xylocaine gel was applied as a local anaesthetic, post-surgically. The surgery lasted, on average, approximately 10 min. The rationale for using the osmotic mini-pumps as a means of drug delivery is twofold: (1) osmotic mini-pumps release the drug at the constant rate for the duration of this chronic treatment study, avoiding the daily fluctuations in drug level, which is also an ideal aim of the clinically used antidepressants; and (2) the substitution of daily i.p. injections with the osmotic mini-pump reduces the stress incurred on the experimental animals. Removing stress as a confounding factor is particularly important given the effects of stress on 5-HT_{1B} receptor functionality [27], and the higher susceptibility of FSL rats to stress.

3.2. Autoradiographic experiment

To avoid the fluctuations of the plasma concentrations of tryptophan and other amino acids, the animals were fasted overnight prior to the day when the autoradiographic experiment was performed. Water was provided ad libitum. To avoid the possible influence of the circadian rhythm on the 5-HT system and measured parameters, the α-MTrp was injected between 11:00 am and 1:00 pm. The anaesthesia was achieved using inhalatory anaesthetic isoflurane (5% concentration for induction and 2% concentration for maintenance). Plastic catheters (PE50) were inserted into the femoral artery (for blood sampling) and the vein (for the tracer injection). Throughout the experiment, the opening of the catheters was maintained by flushing with heparine sodium solution (1000 I.U./mL). The wound was closed with sutures and the lower part of the rat's body was immobilized with a plastic cast to minimize movements which could dislocate the catheters from the vessels. Following the immobilization, the anaesthetic mask was removed and the rats were allowed to recover for at least 2 h prior to the injection of the tracer. Thirty μCi of α-MTrp (specific activity of 55 mCi/mmol; Amersham Bioscience Inc.), dissolved in 1 mL of saline, was infused into the femoral vein over 2 min by an infusion system (Model 55-2226, Harvard Apparatus, Holliston, MA, USA). Blood samples (50 μL) were taken at progressively longer intervals after the infusion of the tracer, on the following schedule (min): 0.5, 1, 1.5, 2, 2.5, 3, 5, 10, 20, 30, 40, 50, 55, 60 (14 samples, 60 min experiment) or 0.5, 1, 1.5, 2, 2.5, 3, 5, 10, 30, 60, 90, 120, 140, 145, 150 (15 samples, 150 min experiment). The blood samples were centrifuged for 3 min at 9300 × *g*, and 20 μL of plasma was used for liquid scintillation

counting to measure the plasma radioactivity needed to calculate the arterial input function. The physiological parameters (pH, pO_2, pCO_2), total, and free levels of plasma Tryptophan were measured from the blood samples (80 μL) withdrawn immediately before and 10 min after the α-MTrp infusion, as well as 10 min before the animals were sacrificed. At 60 or 150 min following the infusion of the tracer, the rats were decapitated using a guillotine. The brains were rapidly removed, frozen in 2-methylbutane (within 2 min following the decapitation) and stored in a deep freezer at −84 °C until being cut. The brains were cut in a Leica CM3000 (Leica Microsystems, Bensheim, Germany) at −20 °C (the thickness of the slices was 30 μm). The slices were then mounted on glass slides and after drying on a hot plate (60 °C), contacted to phosphorous imaging plates (Fuji, Japan) along with ^{14}C-polymer standards (Amersham; calibrated to 30 μm thickness of the brain tissue) for 2 weeks. Following this period, the plates were scanned in a Fuji BAS5000 (Fuji, Japan) scanner.

3.3. The measurement of plasma Trp concentrations

To measure the total and free (non-albumin-bound) Trp concentrations in the plasma, six plasma samples were taken at different time-points (50 min following the infusion in the 60 min experiment and 140 min following the infusion in the 150 min experiment). The samples were shaken on a vortex and spun for 4 min at 9300 rpm (room temperature). For the determination of the total Trp concentration in the plasma, 50 μL of plasma was deproteinized with 25 μL of 20% trichloroacetic acid (TCA). For the measurement of the free Trp concentration in the plasma, an additional 50 μL of plasma was filtered through a Biomax-10 filter (10,000 MW cutoff, Millipore Co., Bedford, MA, USA) spinning at 9300 × *g* for 10 min at 41 °C. Both plasma samples for measuring the free and total Trp were stored in a freezer (−84 °C) until they were analysed using HPLC (high performance liquid chromatography) with fluorescence detection [48].

3.4. Calculation of the 5-HT synthesis rate

The scanned images were digitized on a microcomputer-based image analysis system (MCID; Imaging Research Inc., St. Catharines, Ont., Canada). The system consists of a video camera, a frame grabber and a personal computer. The measured optical densities were converted into tissue radioactivity concentration (nCi/g) in the following way: the optical density of the ^{14}C-standards was plotted as a function of their tissue equivalent and the calibration curve was obtained. The optical density was then converted into the tissue tracer concentration (nCi/g) using a third-order polynomial as a calibration curve. Thirty-seven brain regions of interest were identified using the Rat Brain atlas [39]. The tissue concentration of the tracer was measured bilaterally in 35 regions of interest, with 6–8 bilateral readings per region of interest. The dorsal raphe was partitioned as exemplified in Fig. 1. The tissue radioactivity concentrations were converted into the volume of distribution by dividing the tissue (nCi/g) with the plasma tracer concentrations at the end of the experiment [nCi/mL; C*p(T)], as previously detailed [8]. The rate of 5-HT synthesis (*R*; pmol/g/min) was calculated by converting *K* (slopes obtained from volume of distribution fit as a function of exposure time $\Theta = \int_0^T Cp*(t)\cdot dt / C*p(T)$; here *Cp**(*t*) and *C***p*(*T*) are plasma tracer concentration as a function of time (*t*) and at the end of experiment (*T*), respectively) to K^T (K^T = *K*/LC; division by the lumped constant (LC)) and then multiplying

the calculated K^T by the plasma free (non-protein-bound) Trp (Cp; pmol/mL); $R = CpK/\text{LC} = CpK^T$. The LC for α-MTrp used in the present study was previously measured in vivo and found to be 0.42 ± 0.07 [52], and it is not influenced by drugs [41,52].

3.5. Statistics

STATISTICA 7 software (StatSoft Inc., Tulsa, OK, USA) was used for the statistical analysis of the data. To determine the presence of a region × group interaction, the general linear model of ANOVA was used. The post hoc correction for the multiple comparisons was done according to the Benjamini–Hochberg method [3]. The planned comparisons were used to determine the overall differences in 5-HT synthesis between different groups (FRL-AC-SAL relative to FSL-AC-SAL; FRL-CHR-SAL relative to FSL-CHR-SAL), as well as the effect of acute or chronic treatments in the FRL rats (FRL-AC-SAL relative to FRL-AC-TR; FRL-CHR-SAL relative to FRL-CHR-TR), and the FSL rats (FSL-AC-SAL relative to FSL-AC-TR; FSL-CHR-SAL relative to FSL-CHR-TR). The $p < 0.05$ was taken as significant.

4. Results

The physiological parameters (pO_2, pCO_2, pH) and the free and total tryptophan concentrations in the plasma are shown in Tables 1 and 2. There were no significant differences between the groups in any of the physiological variables measured (Tables 1 and 2), except for the values of pO_2, which were significantly higher in the FRL-CHR-TR rats, relative to the FRL-CHR-SAL rats [$F(1,19) = 4.9$; $p < 0.05$].

A set of autoradiograms exemplifies regional differences in 5-HT synthesis between the different groups in Figs. 2 and 3.

When comparing the FRL-AC-SAL and FSL-AC-SAL groups, three factor ANOVA, followed by the Benjamini–Hochberg correction for multiple comparisons, showed that the 5-HT synthesis rate is significantly different (lower) in 23 out of 35 tested brain regions (66%) in the FSL-AC-SAL group [$F(1,20) = 13.4$; $p < 0.002$); brain region × treatment interaction had $F(34,680) = 40.3$; $p < 0.00001$]. One region, the caudate putamen B medial part, lost significance following Benjamini–Hochberg correction. The differences were most pronounced in the ventral thalamus (−55% in FSL-AC-SAL), followed by the anterior olfactory nucleus (−52%) and substantia nigra B pars compacta (SNC; −44%). The lowest significant differences were found in the raphe magnus (−22% in FSL-AC-SAL) and the frontal cortex (−17%). The only region in which the 5-HT synthesis rate was higher in the FSL rats was the entorhinal cortex (28%), but this difference was not statistically significant (Table 3). The differences between the FRL-SAL and FSL-SAL groups are graphically depicted for a selected number of the brain regions in Fig. 4 to demonstrate the effect.

When comparing the FRL-AC-SAL and FRL-AC-TR groups, three factor ANOVA, followed by the Benjamini–Hochberg correction for multiple comparisons, showed that the 5-HT synthesis rate is significantly different (lower) in the FRL-AC-TR rats, relative to the FRL-AC-SAL group, in 32 out of 35 tested brain regions (91%) [$F(1,21) = 35.7$; $p < 0.001$); brain region × treatment interaction had $F(34,714) = 32.6$; $p < 0.00001$]. Lower 5-HT synthesis

rates in the FSL-AC-TR rats were found in all of the tested regions (with the exception of the parietal cortex, entorhinal cortex and median raphe). Significance in all of the brain regions survived Benjamini–Hochberg corrections (Table 3). The largest decrease was found in the ventral thalamus (−67%), followed by the dorsal hippocampus (−63%), substantia nigra-pars compacta (−60%) and caudate putamen at the level of the globus pallidus (−59%). The lowest significant decreases were found in the nucleus accumbens (−31%) and ventral hippocampus (−34%). Decreases in the raphe regions ranged from −15% (in the median raphe, non-significant) to −55% (raphe pontine) (Table 3). Comparisons of the regional differences are depicted for the subset of the brain regions in Fig. 4 to demonstrate the effect.

When comparing the FSL-AC-SAL and FSL-AC-TR groups, three factor ANOVA showed a significant brain region × group (saline and drug treated groups) interaction [($F(35,735) = 34.6$; $p < 0.00001$)]. After the post hoc Benjamini–Hochberg correction, the differences were significant in 6 out of 35 tested brain regions (17%): the anterior olfactory nucleus, septal nucleus, raphe magnus, median raphe, nucleus accumbens, and medial forebrain bundle. The 5-HT synthesis was significantly higher in the FSL-AC-SAL group, relative to the FSL-AC-TR group, in all of the regions where the difference reached statistical significance, except in the anterior olfactory nucleus. The differences in the ventral thalamus, caudate putamen – medial part, dorsal raphe – ventral part, superior colliculi, and ventral tegmental area lost significance following correction (Table 3).

ANOVA followed by the Benjamini–Hochberg post hoc correction for multiple comparisons showed that the FRL-CHR-SAL group has a greater 5-HT synthesis than the FSL-CHR-SAL group [($F(1,21) = 13.7$; $p < 0.002$); brain region × treatment interaction had ($F(34,714) = 15.8$; $p < 0.00001$)]. The 5-HT synthesis rate is significantly higher in 32 out of 35 brain regions tested (91%) in the FRL-CHR-TR group. The differences were most pronounced in the ventral tegmental area (−68% in FSL-CHR-SAL), followed by the median forebrain bundle (−62%) and sensory cortex (−61%). The lowest significant differences were found in the dorsal raphe – dorsal part (−22% in FSL-CHR-SAL) and ventral hippocampus (−21%). Regional 5-HT synthesis was higher in the FSL-CHR-SAL group in the superior colliculus (27%) and dorsal raphe – ventral part (29%), but these differences were not significant (Table 4). The regional differences are compared for the subset of the brain regions in Fig. 5, exemplifying the stain effect on synthesis in this chronic treatment experiment.

The comparison of the FRL-CHR-SAL and FRL-CHR-TR groups, ANOVA, and the Benjamini–Hochberg post hoc correction, revealed that the 5-HT synthesis rate is significantly different (lower) in 29 out of the 35 brain regions tested (83%) in the FRL-CHR-TR group [($F(1,20) = 12.9$; $p < 0.002$); brain region × treatment interaction had ($F(34,680) = 14.4$; $p < 0.00001$)]. After the post hoc evaluation, significant decreases were found in all of the brain regions except the ventral tegmental area, dorsal raphe – dorsal part, ventral hippocampus, raphe pontine, dorsal raphe – ventral part, and median raphe. There was no region which lost significance after the post hoc correction. The largest decreases in 5-HT synthesis in the FRL-CHR-TR rats were found in the locus coeruleus (−78%), followed by the claustrum (−72%), cingulate cortex (−61%) and frontal cortex (−55%). Significant decreases in the raphe nuclei were −22% in the dorsal raphe – lateral part and

−49% in the raphe magnus. The region with the lowest statistically significant decrease was the dorsal raphe – lateral part (−22%) (Table 4). The regional differences are compared for the subset of the brain regions in Fig. 5 to exemplify the difference in the magnitude of the treatment effect.

The three factor ANOVA for the FSL-CHR-SAL and FSL-CHR-TR group comparisons revealed a significant treatment effect [($F(1,22) = 25.4$; $p < 0.0001$); brain regions × treatment; interaction had ($F(34,748) = 21.4$; $p < 0.0001$)]. The Benjamini–Hochberg post hoc correction for multiple comparisons revealed significant differences in 32 out of the 35 brain regions tested (91%) between the FSL-CHR-TR and FSL-CHR-SAL groups. The brain regions which did not have significant differences in 5-HT syntheses are: the dorsal raphe – ventral part, raphe magnus and superior colliculus. The increases were most pronounced in the ventral tegmental area (308%). The lowest significant increases were found in the dorsal raphe – lateral part (25%) and dorsal raphe – dorsal part (36%) (Table 4). The regional differences are compared for the subset of the brain regions in Fig. 5 to exemplify the difference in the magnitude of the treatment effect.

5. Discussion

In the present study, the acute treatment with the selective and centrally active 5-HT_{1B} agonist CP-94253 resulted in decreased 5-HT synthesis in both the FSL rat model of depression and the FRL controls, although most of the decreases in the FSL group lost significance following the Benjamini–Hochberg correction for multiple comparisons (Table 3). The chronic treatment produced the opposite effect on 5-HT synthesis between those strains (an increase in the FSL and a decrease in the FRL) (Table 4).

The 5-HT synthesis decrease in both the FRL and FSL rats following acute treatment with CP-94253 (Table 3) accords with the effects of the acute treatment with the 5-HT_{1B} agonist, CP-93129, on 5-HT synthesis in the SPD rats [14], which also produced widespread decreases in brain 5-HT synthesis in the terminal regions, with less consistent effects in the raphe nuclei. The microiontoforetic application of the 5-HT_{1B} agonist on hippocampal [1] and raphe [16] neurons decreased the 5-HT release in the SPD rats. Although no such studies have been done in either the FSL or FRL rats, it is possible that the acute stimulation of 5-HT_{1B} receptors in these strains resulted in a decreased release of 5-HT and a consequent end-product inhibition of the 5-HT synthesizing enzyme, Tryptophan hydroxylase (Tph) [38], with decreased 5-HT synthesis as the final outcome. The effects of the acute treatment on 5-HT synthesis in the FRL rats presented here accord with the effects of the sub-chronic (7 day) treatment with the 5-$HT_{1B/1D}$ agonist, CGS 12066B, which decreased 5-HT synthesis in the median raphe and terminal areas, but produced no effect on 5-HT synthesis in the DR [51]. Regarding the relative clinical importance of the 5-HT synthesis in the raphe nuclei vs. the terminal regions, in the currently depressed patients, 5-HT synthesis was significantly decreased in some of the 5-HT terminal regions (cingulate and temporal cortices), relative to age and gender-matched healthy controls, as measured by positron emission tomography [43]. However, the degree of the 5-HT synthesis decrease was not correlated with the severity of the depression symptoms, as measured by the clinically used rating scales. However, in another study, it was found that the rate of 5-HT synthesis

negatively correlates with the Hamilton Depression Rating Scale in the left inferior frontal gyrus (Brodmann area 46) and in the medial frontal gyrus (Brodmann area 10) [11]. The 5-HT synthesis in cell body regions of 5-HT neurons could not have been measured in humans due to the insufficient spatial resolution of positron emission tomography, relative to the size of the raphe nuclei.

The opposite effect of the chronic treatment with the 5-HT_{1B} agonist, CP-94253, on 5-HT synthesis in the FSL model of depression and the FRL controls (Table 4) may be related to the higher density of the 5-HT_{1B} receptors in the FSL rats, relative to both the FRL and SPD rats [30]. The presence of higher densities of 5-HT_{1B} receptors in the FSL rats [30], despite the lack of significant differences in the extracellular 5-HT levels [57] may suggest a different sensitivity of these receptors in the FRL and FSL strains. The 5-HT_{1A} receptors, which also control the synthesis [50] and release of 5-HT as do the 5-HT_{1B} receptors, show lower sensitivity [46] in the FSL rats, relative to the FRL and SPD rats. It should be noted that the autoradiographic method used by [30] could not distinguish the relative contributions of the auto- and heteroreceptor pools of 5-HT_{1B} to the increased density of the 5-HT_{1B} receptors in the FSL rats, as the 5-HT_{1B} terminal auto- and heteroreceptors are found in the same 5-HT projecting regions [4,29] and no ligand, so far, has shown sufficient binding selectivity for one of these receptor pools. Given that 5-HT_{1B} heteroreceptors mediate the release of a number of other neurotransmitters (e.g., GABA, glutamate, acetylcholine), the increased density of the 5-HT_{1B} receptors in the FSL rats may produce a change in the relative influence of these systems on the functioning of the 5-HT system, indirectly affecting 5-HT synthesis. This divergent effect could be amplified by the known differences in the cholinergic (increased sensitivity to cholinergic agonism [33]), GABA-ergic (increased sensitivity to benzodiapines [40]), noradrenergic (increased tissue levels of NA limbic regions [57]) and dopaminergic (decreased 5-HT-induced release of DA [58]) systems in the FSL rats. Several studies have suggested the differences at the second messenger level between the FSL and FRL rats, which could possibly account for the opposite neurochemical or behavioural responses of the FSL and FRL rats to the same neuropharmacologically active compounds. Treatments aimed at the component of the second messenger signalling system in FSL rats (inositol [10]; phosphodiesterase type 5 (PDE5) inhibitor, sildenafil [22]) have corrected the low mobility of FSL rats in the forced swim test, despite the fact that it increased cholinergic signalling, a characteristic considered to be responsible for the sildenafil-induced attenuation of the anti-immobility effect of otherwise effective antidepressants in the forced swim test in SPD rats [20]. Finally Stepie et al. [47] found that 5-HT_{1B} receptors modulate cyclic guanosine mono-phosphate (cGMP) function, a second messenger system which is affected by sildenafil, an inhibitor of phosphodiesterase type 5, a molecule that has shown antidepressant effects in FSL rats [22]. As an interesting parallel to the findings from the present study, the opposite effects of chronic treatment with the selective 5-HT_{1A} partial agonist, buspirone, on 5-HT synthesis, were found in the FRL (decrease) and FSL (increase) rats [31].

In both the FRL and FSL rats, 5-HT synthesis in most parts of the DR was not significantly affected by chronic treatment with CP-94253, while 5-HT synthesis in the MR, another major source of brain 5-HT innervations, was decreased in the FRL rats and increased in the FSL rats. The increased sensitivity of the 5-HT_{1B}-controlled parameter, 5-HT synthesis, in

the median raphe, relative to the dorsal raphe, accords with the suggestion that the 5-HT_{1B} receptors may have a larger influence on 5-HT release from the terminals of the 5-HT neurons projecting from the median raphe, than from the dorsal raphe [6]. The absence of a decrease in 5-HT synthesis in some of the raphe regions could be due to the higher concentrations of Tph in the 5-HT neuron cell bodies [37], which would require higher concentrations of 5-HT to be inhibited. The 5-HT synthesis rates in the dorsal raphe and median raphe have previously been differently affected by chronic treatment with the 5-HT releaser and reuptake blocker, D-fenfluramine [55], but not with SSRIs (fluoxetine [25]).

The effect of the chronic 5-HT_{1B} agonist treatments differed between the FRL (widespread decreases; Table 4) and SPD rats (no significant effect [14]), which have both been used as normal controls in different studies of FSL rats. However, despite the lack of significant effect of chronic treatment with the 5-HT_{1B} agonist, CP-93129, in SPD rats, 5-HT synthesis in most of the examined regions showed a trend toward the decrease in a treated group. The differences in the extent of the blood brain barrier penetrability between CP-94253 and CP-93129, as well as possible differences between the FRL and SPD strains, could be responsible for these outcomes.

The presence of the significant and widespread effects of CP-94253 on 5-HT synthesis following a 14 day treatment in both the FRL and FSL rats suggest the lack of desensitization of the receptors and downstream mechanisms involved in this effect.

The significance of these results regarding the understanding of the 5-HT system regulation in the brains of depressed patients is currently not clear and should be clarified or confirmed by studies examining the efficacy of chronic treatment with CP-94253 in the validated behavioural tests of the antidepressant efficacy in FSL rats, such as the forced swim test. The possibility of the antidepressant efficacy of CP-94253 in the FSL rats is supported by the fact that the chronic treatment of FSL rats with citalopram shows both antidepressant efficacy [35], as well as an increase in 5-HT synthesis in the terminal regions, but a decrease in the raphe [19]. Further, the relationship between the changes in 5-HT synthesis induced by pharmacological treatment and behavioural normalization was corroborated by the findings in the olfactory bulbectomized (OBX) rat model of depression, in which previously increased 5-HT synthesis [53] was decreased through chronic treatment with citalopram [14], using the same dose and length of treatment (14 days, 10 mg/kg/day) which normalized behaviour in OBX rats [23]. Finally, the reduced depression scores in depressed humans chronically treated with citalopram correlated with an increase in 5-HT synthesis in the prefrontal cortex, as measured by positron emission tomography [5].

Lower 5-HT synthesis has been found throughout the brain in FSL rats treated with saline, relative to FRL controls treated with saline, replicating the previous results [15]. The lower 5-HT synthesis in FSL rats may be due to the 5-HT-mediated inhibition of Tph, a 5-HT synthesizing enzyme. Higher tissue concentrations of 5-HT in the FSL rats [56] are presumably accounted for by the increased intracellular concentration of 5-HT, given that the extracellular concentration of 5-HT was not affected. Similar reports on the other monoamine neurotransmitters in FSL rats (NA and DA [57]), as well as the decreased concentration of the vesicular monoamine transporter [45] suggest that decreased

monoamine release may play a role in the pathophysiology of FSL neurochemical, and possibly behavioural, alterations. Therefore, increased intracellular levels of 5-HT in the FSL rats may tonically inhibit 5-HT synthesis. The different effects of treatment with 5-HT_{1B} agonist on 5-HT synthesis in the FSL rat model of depression and the control FRL rats suggest that the interpretation of data obtained from normal rats could be of limited value in deducing the “antidepressant” mechanisms of compounds acting on the 5-HT system. This result is not surprising, given the frequently observed difference in behavioural effects of psychotropic compounds in normal rats and the rat models of depression [23,44].

The purpose of measuring the physiological parameters (pH, pO_2, pCO_2, hematocrit) in the arterial blood during the autoradiographic experiment was to ensure that these parameters were within the physiological range. This was necessary to exclude the possibility that surgical procedure and immobilization had adverse effects on general homeostasis, which might also affect the 5-HT synthesis. There were no significant differences in the physiological parameter values between any of the groups in both the acute and chronic experiment, excluding the significantly higher pO_2 in the FRL-CHR-SAL group, relative to the FRL-CHR-TR group. Although the pO_2 may affect the 5-HT synthesis levels, the mean difference in pO_2 between these two groups was below 5% (Table 2) and not likely to have a significant effect on 5-HT synthesis.

6. Conclusions

Chronic treatment with a selective and centrally active 5-HT_{1B} agonist, CP-94253, has an opposite effect on 5-HT synthesis in the FSL rat model of depression (increase) and the control FRL rats (decrease). The differential effect of chronic 5-HT_{1B} stimulation on 5-HT synthesis in the FSL and FRL rats may be due to the different densities and/or sensitivities of the 5-HT_{1B} auto or heteroreceptor pools.

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Abbreviations

FSL	Flinders Sensitive Line
FRL	Flinders Resistant Line
5-HT	5-hydroxytryptamine
α-MTrp	α-methyl-L-tryptophan
CP-94253	5-propoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-pyrrolo [3,2-b]pyridine hydrochloride
DFP	diisopropyl fluorophosphates

References

1. Adell A, Celada P, Artigas F. The role of 5-HT$_{1B}$ receptors in the regulation of serotonin cell firing and release in the rat brain. Journal of Neurochemistry. 2001; 79:172–182. [PubMed: 11595769]
2. American Psychiatric Association. The Diagnostic Statistical Manual of Mental Disorders. 4. 1994. (DSM-IV)
3. Benjamini Y, Hochberg Y. Controlling the false discovery rate – a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society Series B (Methodological). 1995; 57:289–300.
4. Barnes NM, Sharp T. A review of central 5-HT receptors and their function. Neuropharmacology. 1999; 38:1083–1152. [PubMed: 10462127]
5. Berney A, Nishikawa M, Benkelfat C, Debonnel G, Gobbi G, Diksic M. An index of 5-HT synthesis changes during early antidepressant treatment: alpha[^{11}C]methyl-L-tryptophan PET study. Neurochemistry International. 2008; 52:701–708. [PubMed: 17928105]
6. Blier P, Serrano A, Scatton B. Differential responsiveness of the rat dorsal and median raphe 5-HT systems to 5-HT receptor agonists and pCA. Synapse. 1990; 5:120–133. [PubMed: 2137943]
7. Braw Y, Malkesman O, Dagan M, Bercovich A, Lavi-Avnon Y, Schroeder M, Overstreet DH, Weller A. Anxiety-like behaviors in pre-pubertal rats of the Flinders Sensitive Line (FSL) and Wistar-Kyoto (WKY) animal models of depression. Behavioural Brain Research. 2006; 167:261–269. [PubMed: 16271773]
8. Diksic M, Young SN. Study of the brain serotonergic system with labeled alpha-methyl-L-tryptophan. Journal of Neurochemistry. 2001; 78:1185–1200. [PubMed: 11579128]
9. Dobson CF, Tohyama Y, Diksic M, Hamel E. Effects of acute or chronic administration of anti-migraine drugs sumatriptan and zolmitriptan on serotonin synthesis in the rat brain. Cephalalgia. 2004; 24:2–11.
10. Einat H, Belmaker RH, Zangen A, Overstreet DH, Yadid G. Chronic inositol treatment reduces depression-like immobility of Flinders Sensitive Line rats in the forced swim test. Depression and Anxiety. 2002; 15:148–151. [PubMed: 12001182]
11. Frey BN, Skelin I, Sakai Y, Nishikawa M, Diksic M. Gender differences in α-[^{11}C]MTrp brain trapping, an index of serotonin synthesis, in medication-free individuals with major depressive disorder: a positron emission tomography study. Psychiatry Research: Neuroimaging. 2010; 183:157–166. [PubMed: 20630715]
12. Friedman E, Berman M, Overstreet D. Swim test immobility in a genetic rat model of depression is modified by maternal environment: a cross-foster study. Developmental Psychobiology. 2006; 48:169–177. [PubMed: 16489594]
13. Genud R, Merenlender A, Gispan-Herman I, Maayan R, Weizman A, Yadid G. DHEA lessens depressive-like behaviour via GABA-ergic modulation of the mesolimbic system. Neuropsychopharmacology. 2009; 34:577–584. [PubMed: 18496525]
14. Hasegawa S, Watanabe A, Nishi K, Nguyen K, Diksic M. Selective 5-HT$_{1B}$ receptor agonist reduces serotonin synthesis following acute, and not chronic, drug administration: results of an autoradiographic study. Neurochemistry International. 2005; 46:261–272. [PubMed: 15670643]
15. Hasegawa S, Nishi K, Watanabe A, Overstreet DH, Diksic M. Brain 5-HT synthesis in the Flinders Sensitive Line rat model of depression: an autoradiographic study. Neurochemistry International. 2006; 48:358–366. [PubMed: 16427159]
16. Hopwood SE, Stamford JA. Multiple 5-HT(1) autoreceptor subtypes govern serotonin release in dorsal and median raphé nuclei. Neuropharmacology. 2001; 40:508–519. [PubMed: 11249960]
17. Janowsky DS, Risch C, Parker D, Huey L, Judd L. Increased vulnerability to cholinergic stimulation in affective-disorder patients [proceedings]. Psychopharmacology Bulletin. 1980; 16:29–31.
18. Kanemaru K, Hasegawa S, Nishi K, Diksic M. Acute citalopram has different effects on regional 5-HT synthesis in FSL, FRL, and SDP rats: an autoradiographic evaluation. Brain Research Bulletin. 2008; 77:214–220. [PubMed: 18674602]

19. Kanemaru K, Nishi K, Hasegawa S, Diksic M. Chronic citalopram treatment elevates serotonin synthesis in flinders sensitive and flinders resistant lines of rats, with no significant effect on Sprague-Dawley rats. Neurochemistry International. 2009; 54:363–371. [PubMed: 19418630]
20. Kaster MP, Ferreira PK, Santos AR, Rodrigues AL. Effects of potassium channel inhibitors in the forced swimming test: possible involvement of L-arginine-nitric oxide-soluble guanylate cyclase pathway. Behavioural Brain Research. 2005; 165:204–209. [PubMed: 16122818]
21. Koe KB, Nielsen JA, Macor JE, Heym J. Biochemical and behavioral studies of the 5-HT_{1B} receptor agonist, CP-94253. Drug Development Research. 1992; 26:241–250.
22. Liebenberg N, Harvey BH, Brand L, Brink CB. Antidepressant-like properties of phosphodiesterase type 5 inhibitors and cholinergic dependency in a genetic rat model of depression. Behavioural Pharmacology. 2010; 21:540–547. [PubMed: 20555254]
23. Lucas G, Rymar VV, Du J, Mnie-Filali O, Bisgaard C, Manta S, Lambas-Senas L, Wiborg O, Haddjeri N, Piñeyro G, Sadikot AF, Debonnel G. Serotonin(4) (5-HT(4)) receptor agonists are putative antidepressants with a rapid onset of action. Neuron. 2007; 55:712–725. [PubMed: 17785179]
24. Matrisciano F, Caruso A, Orlando R, Marchiafava M, Bruno V, Battaglia G, Gruber SH, Melchiorri D, Tatarelli R, Girardi P, Mathè AA, Nicoletti F. Defective group-II metaboropic glutamate receptors in the hippocampus of spontaneously depressed rats. Neuropharmacology. 2008; 55:525–531. [PubMed: 18590921]
25. Mück-Seler D, Jevric-Causevic A, Diksic M. Influence of fluoxetine on regional serotonin synthesis in the rat brain. Journal of Neurochemistry. 1996; 67:2434–2442. [PubMed: 8931476]
26. Neumaier JF, Root DC, Hamblin MW. Chronic fluoxetine reduces serotonin transporter mRNA and 5-HT_{1B} mRNA in a sequential manner in the rat dorsal raphe nucleus. Neuropsychopharmacology. 1996; 15:515–522. [PubMed: 8914125]
27. Neumaier JF, Petty F, Kramer GL, Szot P, Hamblin MW. Learned helplessness increases 5-hydroxytryptamine1B receptor mRNA levels in the rat dorsal raphe nucleus. Biological Psychiatry. 1997; 41:668–674. [PubMed: 9066990]
28. Newman ME, Shalom G, Ran A, Gur E, Van de Kar LD. Chronic fluoxetine-induced desensitization of 5-HT_{1A} and 5-HT_{1B} autoreceptors: regional differences and effects of WAY-100635. European Journal of Pharmacology. 2004; 486:25–30. [PubMed: 14751404]
29. Nichols DE, Nichols CD. Serotonin receptors. Chemical Reviews. 2008; 108:1614–1641. [PubMed: 18476671]
30. Nishi K, Kanemaru K, Diksic M. A genetic rat model of depression, Flinders sensitive line, has a lower density of 5-HT(1A) receptors, but a higher density of 5-HT(1B) receptors, compared to control rats. Neurochemistry International. 2009; 54:299–307. [PubMed: 19121358]
31. Nishi K, Kanemaru K, Hasegawa S, Watanabe A, Diksic M. Both acute and chronic buspirone treatments have different effects on regional 5-HT synthesis in Flinders Sensitive Line rats (a rat model of depression) than in control rats. Neurochemistry International. 2009; 54:205–214. [PubMed: 19084042]
32. Overstreet DH, Russell RW, Crocker AD, Schiller GD. Selective breeding for differences in cholinergic function: pre- and postsynaptic mechanisms involved in sensitivity to the anticholinesterase, DFP. Brain Research. 1984; 294:327–332. [PubMed: 6704730]
33. Overstreet DH, Booth RA, Dana R, Risch SC, Janowsky DS. Enhanced elevation of corticosterone following arecoline administration to rats selectively bred for increased cholinergic function. Psychopharmacology (Berl). 1986; 88:129–130. [PubMed: 3080772]
34. Overstreet DH, Russell RW, Booth RA, Jenden DJ. Influence of atropine and N-methyl atropine pretreatments on behavioural and physiological effects of the irreversible muscarinic agonist, BM123, Pharmacology. Biochemistry, and Behavior. 1987; 26:475–481.
35. Overstreet DH, Keeney A, Hogg S. Antidepressant effects of citalopram and CRF receptor antagonist CP-154,526 in a rat model of depression. European Journal of Pharmacology. 2004; 492:195–201. [PubMed: 15178365]
36. Overstreet DH, Friedman E, Mathe AA, Yadid G. The Flinders Sensitive Line rat: a selectively bred putative animal model of depression. Neuroscience and Biobehavioral Reviews. 2005; 29:739–759. [PubMed: 15925699]

37. Park DH, Snyder DW, Joh TH. Postnatal developmental changes of tryptophan hydroxylase activity in serotonergic cell bodies and terminals of rat brain. Brain Research. 1986; 378:183–185. [PubMed: 3742199]
38. Park DH, Stone DM, Baker H, Kim KS, Joh TH. Early induction of rat brain tryptophan hydroxylase (TPH) mRNA following parachlorophenylala-nine (PCPA) treatment. Brain Research Molecular Brain Research. 1994; 22:20–28. [PubMed: 8015380]
39. Paxinos, G., Watson, C. The Rat Brain Atlas in Stereotaxic Coordinates: Compact. 3. Academic Press; New York: 1997.
40. Pepe S, Overstreet DH, Crocker AD. Enhanced benzodiazepine responsiveness in rats with increased cholinergic function, Pharmacology. Biochemistry, and Behavior. 1988; 31:15–19.
41. Pivac N, Diksic M. The lumped constant of α-methyl-L-tryptophan is not influenced by drugs acting through serotonergic system. Neurochemistry International. 2011; 58:826–832. [PubMed: 21414366]
42. Pucilowski O, Overstreet DH. Effect of chronic antidepressant treatment on responses to apomorphine in selectively bred rat strains. Brain Research Bulletin. 1993; 32:471–475. [PubMed: 8221139]
43. Rosa-Neto P, Diksic M, Okazawa H, Leyton M, Ghadirian N, Mzengeza S, Nakai A, Debonnel G, Blier P, Benkelfat C. Measurement of brain regional alpha-[^{11}C]methyl-L-tryptophan trapping as a measure of serotonin synthesis in medication-free patients with major depression. Archives of General Psychiatry. 2004; 61:556–563. [PubMed: 15184235]
44. Sato H, Skelin I, Debonnel G, Diksic M. Chronic buspirone treatment normalizes open field behavior in olfactory bulbectomized rats: assessment with a quantitative autoradiographic evaluation of the 5-HT1A binding sites. Brain Research Bulletin. 2008; 75(5):545–555. [PubMed: 18355630]
45. Schwartz K, Yadid G, Weizman A, Rehavi M. Decreased limbic vesicular monoamine transporter 2 in a genetic rat model of depression. Brain Research. 2003; 965:174–179. [PubMed: 12591135]
46. Shayit M, Yadid G, Overstreet DH, Weller A. 5-HT(1A) receptor subsensitivity in infancy and supersensitivity in adulthood in an animal model of depression. Brain Research. 2003; 980:100–108. [PubMed: 12865163]
47. Stepie A, Chalimoniuk M, Strosznajder J. Serotonin 5HT1B/1D receptor agonists abolish NMDA receptor-evoked enhancement of nitric oxide synthase activity and cGMP concentration in brain cortex slices. Cephalalgia. 1999; 19:859–865. [PubMed: 10668104]
48. Takada A, Grdisa M, Diksic M, Gjedde A, Yamamoto YL. Rapid steady-state analysis of blood–brain transfer of L-Trp in rat, with special reference to the plasma protein binding. Neurochemistry International. 1993; 23:351–359. [PubMed: 8220177]
49. Tatarczynska E, Antkiewicz-Michaluk L, Kłodzińska A, Stachowicz K, Chojnacka-Wójcik E. Antidepressant-like effect of the selective 5-HT$_{1B}$ receptor agonist CP 94253: a possible mechanism of action. European Journal of Pharmacology. 2005; 516:46–50. [PubMed: 15913599]
50. Tohyama Y, Yamane F, Fikre-Merid M, Diksic M. Effects of selective 5-HT1A receptor antagonists on regional serotonin synthesis in the rat brain: an autoradiographic study with alpha-[^{14}C]methyl-L-tryptophan. European Neuropsychopharmacology. 2001; 11:193–202. [PubMed: 11418278]
51. Tohyama Y, Yamane F, Fikre-Merid M, Blier P, Diksic M. Effects of serotine receptors agonists, TFMPP and CGS12066B, on regional serotonin synthesis in the rat brain: an autoradiographic study. Journal of Neurochemistry. 2002; 80:788–798. [PubMed: 11948242]
52. Vanier M, Tsuiki K, Grdisa M, Worsley K, Diksic M. Determination of the lumped constant for the alpha-methyltryptophan method of estimating the rate of serotonin synthesis. Journal of Neurochemistry. 1995; 64:624–635. [PubMed: 7830055]
53. Watanabe A, Tohyama Y, Nguyen KQ, Hasegawa S, Debonnel G, Diksic M. Regional brain serotonin synthesis is increased in the olfactory bulbectomy rat model of depression: an autoradiographic study. Journal of Neurochemistry. 2003; 85:469–475. [PubMed: 12675923]
54. Yadid G, Overstreet DH, Zangen A. Limbic dopaminergic adaptation to a stressful stimulus in a rat model of depression. Brain Research. 2001; 896:43–47. [PubMed: 11277971]

55. Yamane F, Tohyama Y, Diksic M. Acute and chronic D-fenfluramine treatments have different effects on serotonin synthesis rates in the rat brain: an autoradiographic study. Neurochemical Research. 1999; 24:1611–1620. [PubMed: 10591413]

56. Zangen A, Overstreet DH, Yadid G. High serotonin and 5-hydroxyindoleacetic acid levels in limbic brain regions in a rat model of depression: normalization by chronic antidepressant treatment. Journal of Neurochemistry. 1997; 69:2477–2483. [PubMed: 9375680]

57. Zangen A, Overstreet DH, Yadid G. Increased catecholamine levels in specific brain regions of a rat model of depression: normalization by chronic antidepressant treatment. Brain Research. 1999; 824:243–250. [PubMed: 10196455]

58. Zangen A, Nakash R, Overstreet DH, Yadid G. Association between depressive behavior and absence of serotonin-dopamine interaction in the nucleus accumbens. Psychopharmacology (Berl). 2001; 155:434–439. [PubMed: 11441434]

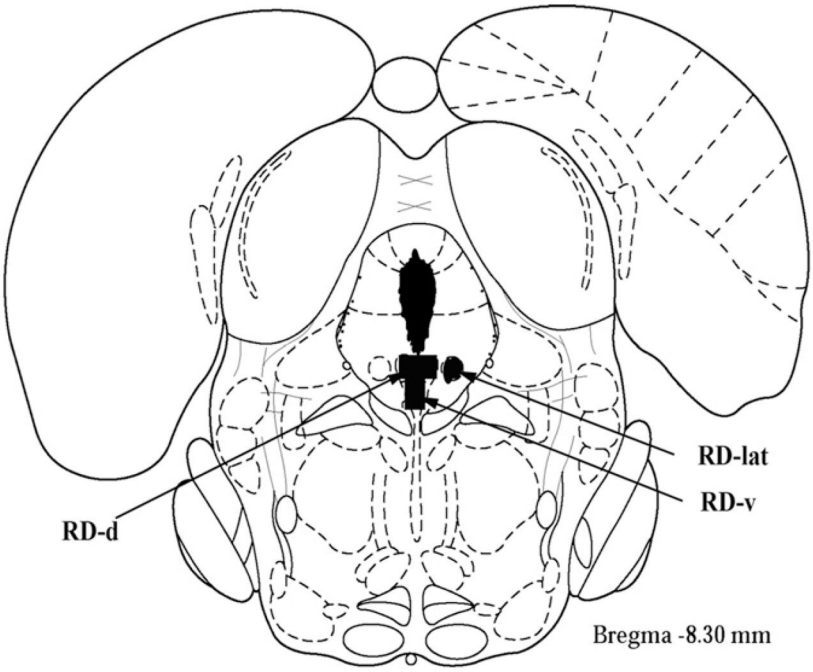

Fig. 1.

A schematic presentation of the regions read as DR-d (dorsal raphe-dorsal part), DR-v (ventral part), and DR-lat (lateral part).

These were adapted from the gross sections provided in [39].

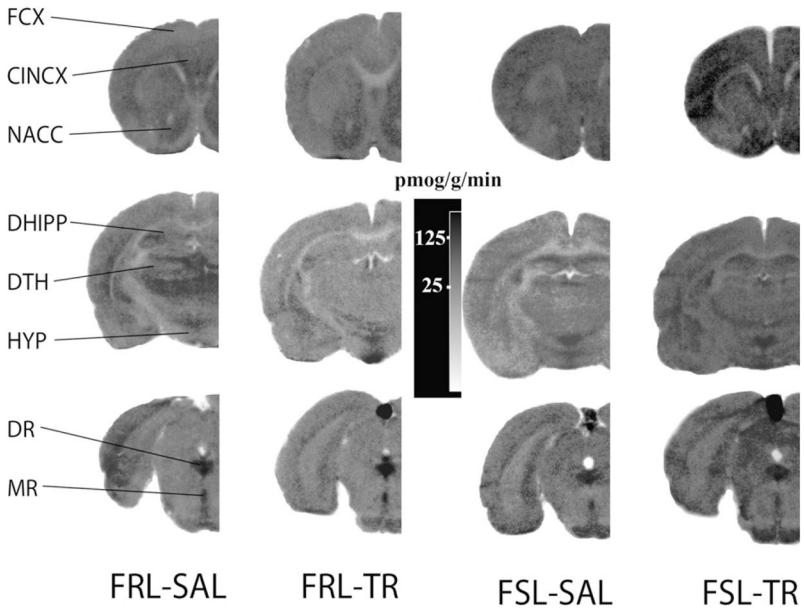

Fig. 2.

A set of autoradiographic images of the coronal sections in acute experiment from the FRL-SAL, FRL-TR, FSL-SAL and FSL-TR groups, illustrating the regional differences in 5-HT synthesis between the experimental groups. The rats were injected i.p. with 5 mg/kg of CP-94253 dissolved in 1 mL/kg of saline (FRL-TR and FSL-TR) or the same volume of saline (FRL-SAL and FSL-SAL). The abbreviations are: CINX, cingulate cortex; DHIP, dorsal hippocampus; DR, dorsal raphe; DTH, dorsal thalamus; HYP, hypothalamus; MR, median raphe; NACC, nucleus accumbens; PB, pineal body.

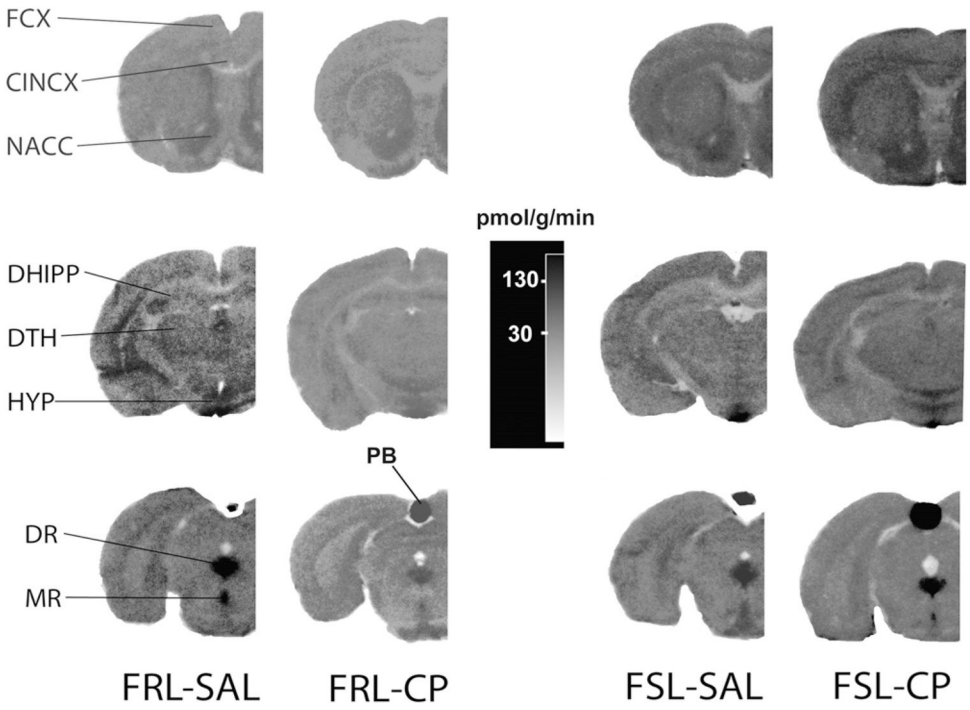

Fig. 3.

A set of autoradiographic images of the coronal sections in chronic experiment from the FRL-SAL, FRL-TR, FSL-SAL and FSL-TR groups. The regional differences in 5-HT synthesis between the experimental groups could be observed, particularly in the high synthesis levels of the raphe nuclei. The rats were treated chronically (14 days) with 5 mg/kg/day of CP-94253 dissolved in 0.12 mL of saline, delivered via a subcutaneously implanted osmotic mini-pump (FRL-TR and FSL-CHR-TR) or the same volume of saline (FRL-SAL and FSL-SAL). The abbreviations are those defined in Fig. 2.

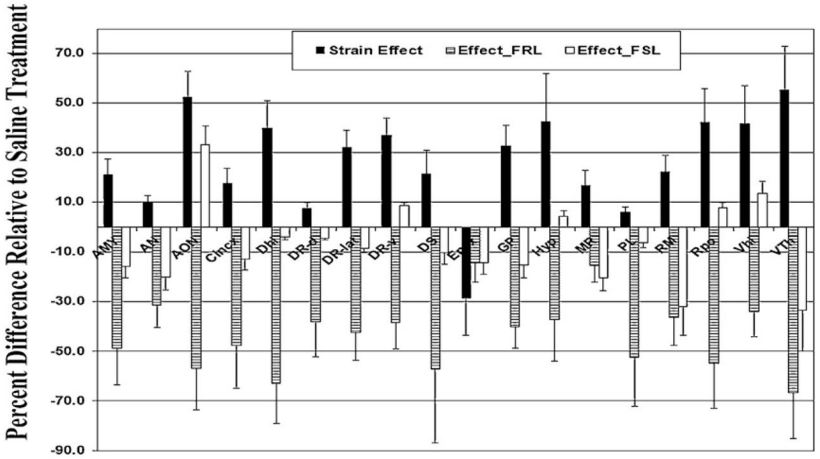

Fig. 4.

The percent differences in 5-HT synthesis rates (%) in the acute experiment between the FRL-AC-SAL and FSL-AC-SAL groups (solid bars; exemplifying the strain effect), between the FRL-SAL and FRL-AC-TR groups (horizontally stripped bars; exemplifying the effect of the treatment in the FRL controls), and between the FSL-AC-SAL and FSL-AC-TR groups (empty bars; exemplifying the effect in FSL rats) are graphically depicted for a selected number of the brain regions.

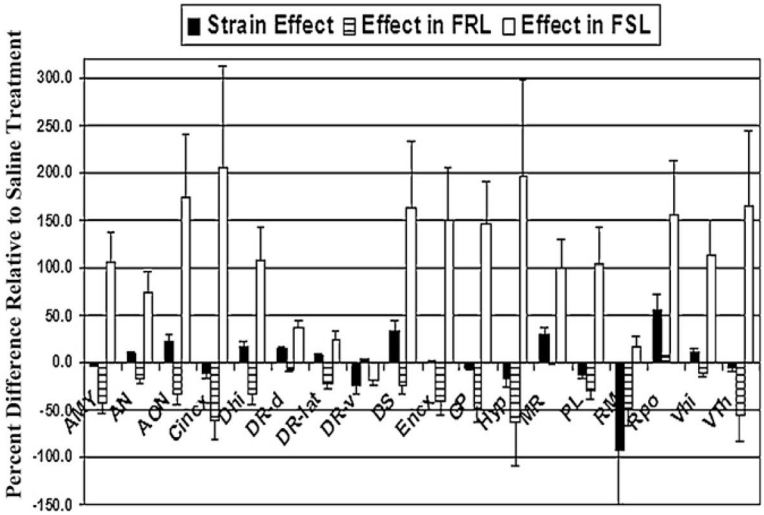

Fig. 5.

The regional differences in 5-HT synthesis (%) in the chronic experiment are compared for the subset of the brain regions, exemplifying the strain effect (solid bars: difference between FRL-CHR-SAL and FSL-CHR-SAL), as well as the effects of the treatment in the FRL (horizontally stripped bars; difference between FRL-CHR-SAL and FRL-CHR-TR) and FSL (empty bars; difference between FSL-CHR-SAL and FSL-CHR-TR) rats.

Table 1

Physiological values in the FRL and FSL rats treated acutely with saline (FRL-AC-SAL; FSL-AC-SAL) or 5 mg/kg of CP-94253 i.p. (FRL-AC-TR; FSL-AC-TR).[a]

	FRL[b]-AC-SAL (*n* = 11)	FRL-AC-TR (*n* = 12)	FSL[c]-AC-SAL (*n* = 11)	FSL-AC-TR (*n* = 11)
Weight (g)	196.1 ± 7.9	197 ± 5.1	206.6 ± 9.0	197.1 ± 11.0
pH	7.42 ± 0.1	7.41 ± 0.02	7.45 ± 0.02	7.42 ± 0.04
pCO_2 (mmHg)	36.3 ± 2.7	38.1 ± 2.5	35.4 ± 1.9	38.0 ± 4.1
pO_2 (mmHg)	99.1 ± 10.0	106.6 ± 8.4	101.6 ± 4.7	115.1 ± 6.2
Free Trp	13.9 ± 6.2	11.6 ± 2.8	11.0 ± 3.0	12.5 ± 2.4
Total Trp	88.1 ± 16.9	87.8 ± 29.0	77.0 ± 16.0	85.4 ± 7.9

[a]The values are presented as mean ± S.D. (standard deviation).

[b]Flinders Resistant Line.

[c]Flinders Sensitive Line.

Table 2

Physiological values in FRL and FSL rats treated for 14 days with saline (FRL-CHR-SAL; FSL-CHR-SAL) or 5 mg/kg/day of CP-94253 (FRL-CHR-TR; FSL-CHR-TR),[a] delivered via subcutaneously implanted osmotic minipump.

	FRL[b]-CHR-SAL (*n* = 11)	FRL-CHR-TR (*n* = 11)	FSL[c]-CHR-SAL (*n* = 12)	FSL-CHR-TR (*n* = 12)
Weight gain (g)	70.7 ± 10.3	65.6 ± 15.9	78.2 ± 11.9	72.3 ± 13.4
pH	7.45 ± 0.03	7.45 ± 0.01	7.44 ± 0.02	7.44 ± 0.03
pCO_2 (mmHg)	35.7 ± 1.8	36.4 ± 3.4	35.1 ± 3.0	34.6 ± 1.8
pO_2 (mmHg)	98.1 ± 7.3[d]	94.3 ± 8.0	101.3 ± 6.3	105.3 ± 10.7
Free Trp (pmol/L)	9.3 ± 1.9	11.2 ± 2.9	8.3 ± 1.4	10.4 ± 2.5
Total Trp (pmol/L)	77.3 ± 15.0	93.9 ± 31.2	59.7 ± 13.1	78.7 ± 14.7

[a] The values are presented as mean ± S.D. (standard deviation).

[b] Flinders Resistant Line.

[c] Flinders Sensitive Line.

[d] Significantly different between the FRL-CHR-SAL and FRL-CHR-TR groups.

Table 3

Regional rates of brain 5-HT synthesis (pmol/g/min) in the FRL and FSL rats following acute treatment with saline (FRL-AC- and FSL-AC-SAL) and CP-94253 (FRL-AC- and FSL-AC-TR groups; 5 mg/kg i.p., 30 min prior to the tracer injection). The values are presented as mean ± S.D. (standard deviation).

Regions	FRL[a]-AC-SAL (*n* = 11)	FRL-AC-TR (*n* = 12)	FSL[b]-AC-SAL (*n* = 11)	FSL-AC-TR (*n* = 11)
Auditory cortex	42.0 ± 11.0	21.9 ± 8.5[*]	37.0 ± 7.1	37.1 ± 9.3
Amygdala	85.0 ± 17.0	43.7 ± 9.6[*]	67.0 ± 14.0[£]	57.0 ± 11.0
Accumbens nucleus	80.0 ± 19.0	54.6 ± 8.5[*]	72.0 ± 12.0	57.0 ± 10.0[¶]
Anterior olfactory nucleus	68.8 ± 6.1	29.7 ± 8.3[*]	32.9 ± 5.9	43.8 ± 5.9[¶]
Cingulate cortex	51.0 ± 13.0	27.0 ± 7.1[*]	42.3 ± 9.5	36.8 ± 9.0
Claustrum	68.0 ± 17.0	28.4 ± 7.9[*]	39.0 ± 11.0[£]	39.0 ± 12.0
Caudate putamen – level of globus pallidus	86.0 ± 18.0	35.0 ± 11.0[*]	48.9 ± 1.0[£]	44.0 ± 11.0
Caudate putamen – lateral part	65.0 ± 16.0	30.4 ± 7.6[*]	44.0 ± 10.0[£]	36.9 ± 9.4
Caudate putamen – medial part	67.0 ± 17.0	43.5 ± 5.4[*]	54.0 ± 12.0[ǂ]	41.0 ± 11.0[§]
Dorsal hippocampus	95.0 ± 17.0	35.2 ± 6.6[*]	57.0 ± 12.0[£]	55.0 ± 11.0
Dorsal raphe – dorsal part	180.0 ± 53.0	111.0 ± 25.0[*]	166.0 ± 16.0	159.0 ± 14.0
Dorsal raphe – lateral part	199.0 ± 26.0	115.0 ± 26.0[*]	135.0 ± 24.0[£]	124.0 ± 16.0
Dorsal raphe – ventral part	307.0 ± 50.0	190.0 ± 43.0[*]	194.0 ± 19.0[£]	211.0 ± 17.0[§]
Dorsal subiculum	54.0 ± 18.0	23.4 ± 9.8[*]	43.0 ± 12.0	38.0 ± 14.0
Entorhinal cortex	41.0 ± 20.0	35.5 ± 9.1	53.0 ± 11.0	46.0 ± 11.0
Frontal cortex	51.0 ± 11.0	22.7 ± 7.1[*]	37.7 ± 9.5[£]	35.0 ± 11.0
Globus pallidus	58.1 ± 9.0	34.9 ± 5.3[*]	39.2 ± 8.4[£]	33.3 ± 9.1
Hypothalamus	78.0 ± 23.0	47.0 ± 16.0[*]	45.0 ± 15.0[£]	46.0 ± 18.0
Median forebrain bundle	61.0 ± 20.0	31.4 ± 6.7[*]	43.0 ± 13.0[£]	30.5 ± 8.9[¶]
Medial geniculate	52.0 ± 14.0	27.1 ± 7.1[*]	37.5 ± 9.6[£]	32.3 ± 9.9
Median raphe	116.0 ± 39.0	98.0 ± 25.0	96.0 ± 11.0	77.0 ± 17.0[¶]
Parietal cortex	43.0 ± 14.0	37.0 ± 13.0	37.3 ± 9.3	35.0 ± 11.0
Prefrontal cortex	52.0 ± 15.0	24.7 ± 6.2[*]	49.0 ± 11.0	45.9 ± 9.0
Raphe magnus	74.0 ± 12.0	47.0 ± 13.0[*]	58.0 ± 15.0[£]	39.0 ± 10.0[¶]
Raphe pontine	142.0 ± 35.0	64.0 ± 15.0[*]	82.0 ± 17.0[£]	88.0 ± 15.0
Superior colliculus	51.5 ± 8.4	24.7 ± 6.0[*]	38.0 ± 12.0[£]	29.8 ± 9.0[§]
Sensory cortex	45.0 ± 14.0	19.9 ± 7.9[*]	35.0 ± 12.0	32.0 ± 11.0
Septal nucleus	58.2 ± 8.0	30.4 ± 5.3[*]	48.0 ± 10.0[£]	31.1 ± 9.1[¶]
Sensory-motor cortex	53.0 ± 9.9	23.4 ± 6.8[*]	36.0 ± 9.1[£]	33.0 ± 11.0
Substantia nigra – core	67.0 ± 12.0	26.7 ± 6.9[*]	37.2 ± 9.7[£]	35.7 ± 8.7
Substantia nigra – reticularis	41.0 ± 11.0	19.9 ± 6.3[*]	26.5 ± 7.8[£]	27.7 ± 8.9

Regions	FRL[a]-AC-SAL (*n* = 11)	FRL-AC-TR (*n* = 12)	FSL[b]-AC-SAL (*n* = 11)	FSL-AC-TR (*n* = 11)
Visual cortex	59.0 ± 18.0	24.3 ± 9.4[*]	40.7 ± 8.9[£]	39.0 ± 11.0
Ventral hippocampus	72.0 ± 14.0	48.0 ± 11.0[*]	42.0 ± 13.0[£]	48.0 ± 8.2
Ventral tegmental area	69.0 ± 11.0	36.9 ± 9.9	46.0 ± 12.0[£]	37.4 ± 7.0[§]
Ventral thalamus	82.0 ± 7.9	27.4 ± 7.2[*]	37.0 ± 11.0[£]	24.4 ± 9.6[§]

[a] Flinders Resistant Line.

[b] Flinders Sensitive Line.

[*] Significantly different 5-HT synthesis rates between the FRL-AC-SAL and FRL-AC-TR rats ($p < 0.05$).

[¶] Significantly different 5-HT synthesis rates between the FSL-AC-SAL and FSL-AC-TR rats ($p < 0.05$).

[£] Significantly different 5-HT synthesis rates between the FRL-AC-SAL and FSL-AC-SAL rats ($p < 0.05$).

[ǂ] Significance for the FRL-AC-SAL and FSL-AC-SAL comparison, but the significance is lost following Benjamini–Hochberg correction for multiple comparisons.

[§] Significance for the FSL-AC-SAL and FRL-AC-TR comparison, but the significance is lost following Benjamini–Hochberg correction for multiple comparisons.

Table 4

Regional rates of brain 5-HT synthesis (pmol/g/min) in the FRL and FSL rats following chronic treatment with saline (FRL-CHR- and FSL-CHR-SAL) and CP-94253 (FRL-CHR-and FSL-CHR-TR groups; 5 mg/kg/day for 14 days, delivered by subcutaneously implanted osmotic minipump) on the rate of 5-HT synthesis (pmol/g/min) in the rat brain. The values are presented as mean ± S.D. (standard deviation).

Regions	FRL[a]-CHR-SAL (*n* = 11)	FRL-CHR-TR (*n* = 11)	FSL[b]-CHR-SAL (*n* = 12)	FSL-CHR-TR (*n* = 12)
Auditory cortex	33.7 ± 8.2	14.5 ± 5.7[*]	14.7 ± 5.7[£]	46.9 ± 15.2[¶]
Amygdala	57.4 ± 10.3	33.3 ± 6.0[*]	34.4 ± 6.4[£]	70.6 ± 18.0[¶]
Accumbens nucleus	59.2 ± 11.7	49.1 ± 7.6[*]	44.3 ± 5.8[£]	77.1 ± 20.0[¶]
Anterior olfactory nucleus	36.2 ± 6.4	24.1 ± 6.2[*]	19.0 ± 5.9[£]	52.0 ± 12.8[¶]
Cingulate cortex	39.4 ± 9.7	15.3 ± 3.2[*]	17.1 ± 7.1[£]	52.2 ± 16.8[¶]
Claustrum	37.7 ± 10.4	10.7 ± 4.3[*]	18.1 ± 6.9[£]	52.4 ± 17.5[¶]
Caudate putamen – level of globus pallidus	50.7 ± 11.4	33.0 ± 5.2[*]	33.7 ± 4.8[£]	64.9 ± 17.3[¶]
Caudate putamen – lateral part	42.0 ± 10.2	28.8 ± 4.5[*]	24.8 ± 4.9[£]	60.3 ± 16.6[¶]
Caudate putamen – medial part	53.7 ± 10.3	32.7 ± 6.5[*]	28.5 ± 5.7[£]	71.0 ± 19.2[¶]
Dorsal hippocampus	57.5 ± 12.9	37.8 ± 6.5[*]	31.2 ± 4.9[£]	64.7 ± 18.8[¶]
Dorsal raphe – dorsal part	132.4 ± 28.5	122.5 ± 9.8	103.6 ± 11.1[£]	140.8 ± 30.5[¶]
Dorsal raphe – lateral part	130.1 ± 27.0	101.2 ± 12.2[*]	93.5 ± 19.5[£]	116.9 ± 28.6[¶]
Dorsal raphe – ventral part	172.6 ± 35.9	177.9 ± 24.1	222.7 ± 63.8[£]	181.3 ± 38.5
Dorsal subiculum	41.3 ± 13.1	31.0 ± 6.4[*]	20.8 ± 5.4[£]	54.7 ± 19.0[¶]
Entorhinal cortex	41.1 ± 8.4	24.4 ± 6.9[*]	23.9 ± 6.0[£]	59.8 ± 16.5[¶]
Frontal cortex	34.7 ± 8.7	15.7 ± 4.1[*]	18.0 ± 6.2[£]	44.7 ± 14.9[¶]
Globus pallidus	40.7 ± 8.8	21.5 ± 5.6[*]	22.7 ± 3.3[£]	55.8 ± 14.3[¶]
Hypothalamus	48.3 ± 10.7	17.6 ± 11.6[*]	20.3 ± 6.8[£]	60.1 ± 22.8[¶]
Median forebrain bundle	39.6 ± 10.2	22.8 ± 6.1[*]	15.0 ± 7.0[£]	50.1 ± 17.7[¶]
Medial geniculate	35.0 ± 8.8	19.7 ± 6.1[*]	22.4 ± 6.8[£]	46.3 ± 16.8[¶]
Median raphe	84.6 ± 20.7	84.6 ± 15.0	60.0 ± 14.8[£]	120.2 ± 21.8[¶]
Parietal cortex	28.8 ± 8.2	11.3 ± 4.7[*]	16.1 ± 5.3[£]	44.1 ± 14.8[¶]
Prefrontal cortex	33.7 ± 8.4	23.8 ± 3.5[*]	27.0 ± 6.4[£,§]	54.9 ± 16.4[¶]
Raphe magnus	52.5 ± 13.4	26.8 ± 7.3[*]	51.8 ± 28.2	60.9 ± 16.3
Raphe pontine	74.2 ± 14.6	78.5 ± 15.0	34.5 ± 7.8[£]	88.5 ± 25.3[¶]
Superior colliculus	29.9 ± 9.4	13.9 ± 4.4[*]	37.9 ± 15.2	44.2 ± 17.9
Sensory cortex	30.9 ± 9.2	13.5 ± 4.4[*]	12.1 ± 5.4[£]	43.2 ± 16.0[¶]
Septal nucleus	35.9 ± 9.9	21.4 ± 4.4[*]	20.5 ± 5.6[£]	52.3 ± 15.1[¶]
Sensory-motor cortex	29.6 ± 7.6	19.1 ± 4.8[*]	12.5 ± 5.3[£]	45.7 ± 16.7[¶]
Substantia nigra – core	33.0 ± 8.1	24.7 ± 8.0[*]	16.6 ± 6.6[£]	46.5 ± 16.2[¶]

Regions	FRL[a]-CHR-SAL (*n* = 11)	FRL-CHR-TR (*n* = 11)	FSL[b]-CHR-SAL (*n* = 12)	FSL-CHR-TR (*n* = 12)
Substantia nigra – reticularis	25.9 ± 7.8	17.1 ± 3.8[*]	19.6 ± 7.5	39.1 ± 13.8[¶]
Visual cortex	36.1 ± 8.8	14.6 ± 4.7[*]	19.6 ± 7.5[£]	53.2 ± 16.3[¶]
Ventral hippocampus	36.9 ± 10.7	32.9 ± 6.7	29.0 ± 5.8[£]	61.7 ± 16.9[¶]
Ventral tegmental area	36.8 ± 10.7	32.6 ± 6.7	11.6 ± 6.7[£]	47.3 ± 16.5[¶]
Ventral thalamus	43.8 ± 11.2	19.8 ± 8.8[*]	20.9 ± 6.8[£]	55.3 ± 19.7[¶]

[a] Flinders Resistant Line.

[b] Flinders Sensitive Line.

[*] Significantly different 5-HT synthesis rate between the FRL-CHR-SAL and FRL-CHR-TR rats ($p < 0.05$).

[¶] Significantly different 5-HT synthesis rate between the FSL-CHR-SAL and FSL-CHR-TR rats ($p < 0.05$).

[£] Significantly different 5-HT synthesis rate between the FRL-CHR-SAL and FSL-CHR-SAL rats ($p < 0.05$).

[§] Lost significance following Benjamini-Hochberg correction for multiple comparisons.