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Acute citalopram has different effects on regional 5-HT synthesis in FSL, FRL, and SDP rats; an autoradiographic evaluation

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Abstract

In this study, we measured the effect of an acute treatment of citalopram on 5-HT synthesis in a genetic rat model of depression, the Flinders Sensitive Line (FSL) rats, their counterparts, the Flinders Resistant Line (FRL) rats, and outbred Sprague-Dawley (SPD) rats, using the α -[¹⁴C]methyl-L-tryptophan (α -MTrp) autoradiographic method. A comparison of 5-HT synthesis in the FSL rats treated with citalopram (FSL-CTP) and those treated with saline (FSL-SAL) indicate that citalopram reduces global 5-HT synthesis in the FSL rats, as well as in all the brain areas investigated. The reduced synthesis was also observed in the dorsal raphe nucleus (DR) and the median raphe nucleus (MR). The comparison of the synthesis between the citalopram treated SPD rats (SPD-CTP) and the saline treated SPD rats (SPD-SAL) revealed a global increase of 5-HT synthesis in the SPD-CTP group, as well as an increase in some terminal areas, but a reduction in the DR and the MR. In contrast to the reduction throughout the brain in the FSL rats, the FRL rats treated with citalopram (FRL-CTP), when compared to the saline group (FRL-SAL), showed a global increase of 5-HT synthesis, as well as in most of the terminal areas and in the DR and the MR. The reduction of 5-HT synthesis throughout the brain in the FSL rats is likely a result of reported supersensitivity of the 5-HT_{1A} receptors. Comparing changes in the SPD, FRL, and FSL rats treated with citalopram to their respective controls (saline treated rats), the FSL rats treated acutely with citalopram were the only rats that exhibited lower 5-HT synthesis rates in all of the limbic areas, the basal ganglia, and the neocortices. This may be related to the pathophysiological basis of depressive characteristics in FSL rats. The citalopram treatment produced unexpected results in the FRL rats: 5-HT synthesis was elevated not only in most of the terminal areas, but also in the cell body areas, the DR and MR. The increase of 5-HT synthesis throughout the brain in the FRL rats is likely a result of the reported subsensitivity of the 5-HT_{1A} receptors, and possibly other sites through which 5-HT synthesis could be controlled (e.g., 5-HT_{1B}). In addition differences in intracellular signaling could be at least in part responsible for these differences.

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Keywords

tracer; depression model; autoradiography; antidepressant; α -[^{14}C]methyl-L-tryptophan

Introduction

The exact causes of depressive disorders and the modes of action of antidepressant drugs are still not clear. Effective treatments for depression require a full understanding of the neurochemical basis of the disease and the changes produced by such treatments. Although the antidepressant, citalopram, is widely used for the treatment of depressed patients, the mechanisms of its therapeutic action are still not fully understood. Because antidepressant treatments do not affect the mood and behavior of non-depressed subjects, the results obtained with healthy volunteers or normal rats subjected to such treatments may have limited relevance to the pathophysiology of the disease. It is difficult to explain depression and the action of antidepressants from a neurotransmission perspective as neurotransmitters can be released non-synaptically [20,50]. In addition, it has been shown that noradrenergic neurons can take up 5-HT (5-hydroxytryptamine; serotonin) [51]. Along these lines, a new strategy in the treatment of depression focusing on the enhancement of non-synaptic interactions has been proposed [20].

It is well accepted that the administration of acute serotonin (5-HT) selective reuptake inhibitors (SSRI) results in a decreased firing rate [21] and a reduced release of 5-HT at the nerve terminals [14]. It has been shown that the somatodendritic 5-HT_{1A} receptors are responsible for these effects, as selective 5-HT_{1A} receptor antagonists effectively counteract the decreases [1,15]. However, it is not yet clear how the acute administration of citalopram affects brain regional 5-HT synthesis. Several sets of data suggest that 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A/C}, 5-HT₃, and 5-HT₄ receptor antagonists (*e.g.*, WAY-100,635, GR127935, ketanserin, ondansertone, and RS-39604) cannot modify the effect of acute citalopram treatment on 5-HT synthesis, as determined by measuring the accumulation of 5-hydroxytryptophan following the inhibition of aromatic amino acid decarboxylase with m-hydroxybenzylhydrazine [23, 45]. Nevertheless, it has been demonstrated that pindolol, a partial agonist for the 5-HT_{1A} receptor [40], could counteract the decreases in 5-HT synthesis rates induced by the acute administration of citalopram [31]. In a human study augmentation of citalopram treatment with pindolol had influence on the regional synthesis of 5-HT [3]. It is possible that the use of different methods employed in the above studies could be responsible, in part, for somewhat different findings, but pindolol has a somewhat different spectrum of action. In addition it was shown that 5-HT_{1A} agonists can influence behavior in animal models of anxiety and depression, possibly suggesting an important role of these sites in depression and anxiety [6].

FSL (Flinders Sensitive Line) rats represent a unique genetic animal model of a subset of human depression [33]. FSL and FRL (Flinders Resistant Line) rats were selectively bred from Sprague-Dawley (SPD) rats to exhibit behavioral supersensitivity (FSL) and subsensitivity (FRL) to the anticholinesterase agent, disopropyl fluorophosphatase [38,41]. FSL rats manifest reduced locomotion, increased anhedonia in response to chronic mild stress, an increased amount of rapid eye movement (REM) sleep, reduction in REM-sleep onset, cognitive difficulties, and reduced body weight [33]. The chronic treatment of FSL rats with antidepressants, such as desipramine, imipramine, sertraline, fluoxetine, and citalopram, diminishes behavioral manifestations related to human depression [35]. A large majority of these comparisons were done relative to the effects in FRL rats. Thus, the FSL rats are regarded as an appropriate and valuable experimental model for studying the neurochemical mechanisms involved in depressive disorders and the mode of action of antidepressants [33,55].

A number of serotonergic abnormalities in FSL rats have been reported. The tissue concentration of 5-HT and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA), were three- to eight-fold higher in the nucleus accumbens, prefrontal cortex, hippocampus, and hypothalamus of the FSL rats, when compared to the SPD rats [58]. This was not the case, however, in the dorsal raphe (DRN), median raphe (MRN), and striatum of the FSL rats, when compared to the SPD rats [58]. In a microdialysis study, the level of extracellular 5-HT in the nucleus accumbens of FSL rats was increased more by acute paroxetine (a SSRI) treatment than in the SPD rats [55]. Furthermore, 5-HT_{1A} receptor supersensitivity in the FSL rats has been reported. 5-HT_{1A} agonists (8-OH-DPAT) induced a greater hypothermic effect on FSL rats than SPD or FRL rats [34,37,44]. In addition to the supersensitivity, an increase in the 5HT_{1A} receptor density, compared to the FRL rats, was found in the hippocampus membrane of the FSL rats [43]. It was also shown that chronic treatment with citalopram has antidepressant effect in FSL rats relative to the FRL rats [36] but this behavioral study did not report any biochemical changes produced by citalopram.

In this study, the effect of the acute treatment of citalopram on 5-HT synthesis in the FSL, FRL and SPD rats, was determined using the α -[¹⁴C]methyl-L-tryptophan (α [¹⁴C]MTrp) autoradiographic method. Because any treatment with antidepressants begins with an acute treatment, it was important to understand the responses of the different components of the brain serotonergic system to such treatment. In an attempt to obtain a better understanding of the modulation of regional serotonin synthesis, we studied brain 5-HT synthesis in the animal model of depression, FSL rats, and used both the SPD and FRL rats as the controls. Because it is the case that in many studies of different behavioral and neurochemical parameters in FSL rats, either FRL or SPD rats were used as the control animals, it was important to evaluate if acute citalopram would produce a similar effect on one of the most important parameters of serotonergic neurotransmission, 5-HT synthesis [27]. The study in FSL rats is particularly important because the action of antidepressants is likely different in “normal” and “depressive” (FSL) animals. This has been demonstrated in studies of several behavioral parameters [5,11] and 5-HT synthesis in olfactory bulbectomized rats [13,53], another rat model of depression.

Materials and Methods

Animals

Male FSL, FRL and SPD rats, weighing between 190–230g, were used in these experiments. The rats were divided into the following groups: FSL citalopram (FSL-CTP; N=12) and saline (FSL-SAL; N=19), FRL citalopram (FRL-CTP; N=14) and saline (FRL-SAL; N=11), and SPD citalopram (SPD-CTP; N=17) and saline (SPD-SAL; N=14). The FSL and FRL rats were obtained from breeding colonies maintained at the Montreal Neurological Institute from virus free breeds kindly supplied by Dr. D. Overstreet (Center for Alcohol Studies, University of North Carolina, Chapel Hill, NC 27599–7178, U.S.A.). The rats were housed in the animal facility (at a room temperature of 22°C with a 7:00 a.m. to 7:00 p.m. lights on-off cycle) and had free access to food and tap water. The SPD rats were obtained from the Chares River Canada and were housed for at least 3 days before the experiment.

Experimental procedure

The detailed experimental procedure has been described in previous publications [12,13,26, 53]. Therefore, only a summary will be provided. The rats 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 experiment. The tracer was always injected between 12:00 PM and 2:00 PM, and all of the rats were sacrificed between 2:00 PM and 4:00 PM. 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 femoral artery and vein were cannulated with plastic catheters under 1–2% of halothane 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 60 W lamp. Ninety minutes following the cannulation surgery, the treatment group was injected intraperitoneally with citalopram solution (10 mg/kg dissolved in saline; 2 ml/kg) and the control group was injected with saline (2 ml/kg). The rats were randomly assigned to the treatment or control (saline treated) groups. Thirty minutes later, 25–30 μCi of α -[^{14}C]MTrp in 1 ml of saline was injected through a catheter into the femoral vein over a two minute period by an injection pump.

Arterial blood samples were taken at progressively increasing intervals, starting from the beginning of the tracer injection to the decapitation of the rats. Following centrifugation for 3 minutes at 9,300g, 20 μL of plasma was taken and the ^{14}C -radioactivity was measured by liquid scintillation counting to obtain the input function. Six plasma samples (0.05 mL each) were taken at different times for the determination of the total and free (non-albumin-bound) tryptophan (Trp) concentration using a previously described procedure [46]. The physiological parameters of the arterial blood (PO_2 , PCO_2 , pH, and hematocrit) were also measured at least twice in each experiment. After the decapitation, the brains were rapidly removed and frozen in isopentane (-25°C).

The brain sections (30 μm thick) were obtained with a cryotome and contacted to X-ray film along with [^{14}C]methyl methacrylate standards (American Radiolabel Chemicals, Inc., St. Louis, MO recalibrated to the Amersham standards) in X-ray cassettes for 3–5 weeks to obtain the autoradiograms. The resulting images on the X-ray film were analyzed, using a microcomputer-based image analyzing system (MCID/M4-Image Analysis System, Imaging Research, St Catharines, ON, Canada), as well as tissue equivalent calibrated standards.

The 5-HT synthesis rates R [$\text{pmol}/(\text{g min})$] were calculated from the trapping constant of α -[^{14}C]MTrp [K^* ; $\text{ml}/(\text{g min})$] and the plasma concentration of the plasma free (non-protein-bound) Trp, where C_p (in pmol/mL) as $R = C_p \cdot K^* / \text{LC}$ [26]. The LC (lumped constant) measured *in vivo* in the rat brain was estimated to be 0.42 ± 0.07 [49].

Statistical Analysis—Statistical evaluations of the physiological group data were performed by an analysis of variance (ANOVA). The effect of treatment in each of the strain of rats was evaluated by a two-factor ANOVA (factor-1=saline/citalopram (group); factor-2=brain region). The main effects of each of the factors and the interaction between the factors (group*region) were evaluated for significance. Significance in factor-1 would indicate a significant effect of citalopram, while significance in factor-2 would indicate significant differences between different brain regions. A post hoc evaluation was performed by carrying out planned comparisons, as we were interested in the differences in a particular brain region only, and not the differences between brain regions. In all cases, the statistical significance was defined as $p < 0.05$.

Results

There were no significant differences in the physiological parameters (*e.g.*, pH, PaCO_2 , PaO_2) and hematocrit between the control and treatment group (Table 1). There was no significant difference in the plasma free or total tryptophan concentration between the control (saline treated) and treatment groups in any strain of rats. However, the free and total tryptophan in the SPD rats was significantly higher than in the FSL and FRL rats, as previously reported for naïve animals [12].

A set of representative autoradiographic images of 5-HT synthesis rates is shown in Fig. 1. It can be seen that there is greater 5-HT synthesis in regions known to contain serotonergic cell bodies (*e.g.*, dorsal raphe (DR)) or the brain areas receiving dense serotonergic projections (*e.g.*, accumbens (AC), median caudate (CM)). 5-HT synthesis rates are provided in Table 2 for various brain structures for each strain of rats.

Because the SPD rats were considered as the overall control groups, the effect of acute citalopram will be described first in this strain. The two-factor ANOVA analysis indicated no overall significant difference (factor-1 (group) main effect) between the SPD-SAL and SPD-CTP groups ($F(1,870)=3.2$; $p>0.05$). There were significant effects of factor-2 (regions) ($F(29,870)=347$; $p<0.001$) and group-region interaction (group*region; $F(29,870)=9.56$; $p<0.001$). Because of group*region significant interaction planned comparisons as a post hoc evaluation were performed to test for significant differences between individual brain regions between SPD-SAL and SPD-CIT groups. This post hoc analysis revealed that the citalopram treated SPD rats (SPD-CTP) had significantly lower 5-HT synthesis than the saline treated SPD rats (SPD-SAL) (Table 2) in the areas containing serotonergic cell body areas (*e.g.*, DR; -17%, MR; -18%). In the terminal areas, the SPD-CTP group showed significant increases in 5-HT synthesis in several areas (anterior olfactory nucleus-median (AONM), 34%; accumbens nucleus (AC), 10%; cingulate cortex (Cincx), 21%; frontal cortex (FCx), 31%; sensory-motor cortex (SMCx), 30%; globus pallidus (GP), 10%; lateral geniculate body, 13%). In the other structures, there were no significant changes in 5-HT synthesis.

In the FSL rats, the two-factor ANOVA indicated a highly significant overall influence of the citalopram treatment (group effect; $F(1,990)=924$; $p<0.001$). The effect for factor-2 (region) was also significant ($F(29,900)=102.4$; $p<0.001$), as was the region*group interaction ($F(29,900)=2.09$; $p<0.001$). The planned comparison showed that there were significant differences in all of the brain regions investigated (Table 2). The FSL-CTP (citalopram treated FSL) rats had lower 5-HT synthesis than the FSL-SAL (saline injected FSL) rats in all brain regions evaluated (Table 2), with large reductions in the cell body areas (DR, 26%; MR, 30%; pontine raphe (PR), 38%). In the terminal areas, significant decreases were observed in the limbic structures (*e.g.*, hypothalamus (Hyp), 42%; amygdala (Amy), 26%; Cincx, 30%; AC, 28%; AONM, 23%), cortices (*e.g.*, frontal (FCx), 33%; parietal (PCx), 29%; SMCx, 44%), and some dopaminergic/noradrenergic regions (*e.g.*, substantia nigra compacta (SNc), 40%; substantia nigra reticulata (SNr), 46%; locus coeruleus (LC), 49%; caudate-lateral (CL), 38%; caudate-medial (CM), 36%). As mentioned above, the reduction in other regions was also significant (Table 2).

The two-way ANOVA analysis of the FRL groups indicated a highly significant effect of citalopram on 5-HT synthesis (group; $F(1,690)=1070$; $p<0.001$). The region effect (factor-2) was also highly significant ($F(29,690)=115.7$; $p<0.001$) with a significant region*group interaction ($F(29,690)=1.51$; $p<0.05$). The regions in which significant effects were found after planned comparisons are identified in Table 2. In contrast to the FSL rats and, to some extent, the SPD rats, the FRL rats treated with citalopram (FRL-CTP), compared with the saline group (FRL-SAL), showed a highly significant increase in overall 5-HT synthesis rates (Table 2). This was also the case in the majority of brain regions investigated. Acute citalopram produced (Table 2) significantly higher 5-HT synthesis rates in the cell body areas (DR, 17%; MR, 21%; PR, 42%) with large increases in some terminal areas (*e.g.*, dorsal and ventral thalamus (119% and 125%, respectively), dorsal and ventral hypothalamus (119% and 74%, respectively), amygdala; 112%, AONM; 104%, AC; 72%), Cincx; 100%).

Discussion

The data clearly demonstrate that acute citalopram has a rather different effect on global and regional 5-HT synthesis rates in normal rats (SPD rats) (no main effect) than in “depressed” FSL rats (Table 2). Even a greater effect was found after treatment with citalopram in the FRL rats, the strain very often used as controls in studies with FSL rats [18,35,54,57]. In that case, the citalopram produced a significant elevation in both global and regional 5-HT synthesis (Table 2).

Free and total Trp concentration in the plasma of the SPD rats was significantly higher than in the FSL and FRL rats (Table 1). Similarly, higher plasma Trp concentrations were observed in the naïve SPD rats, relative to the FSL and FRL rats [12]. It should be noted that 5-HT synthesis, as calculated by the model used here, is, to some extent, a function of plasma free Trp [4,7,26,28,30,42,49]. Therefore, 5-HT synthesis rates, relative to the other two strains, in both the SPD-CTP and SPD-SAL groups were more affected by free Trp than the other four groups. Therefore, when one compares the results between the treatment and respective control groups for each strain of rats, the plasma free Trp should not influence the conclusions.

In the SPD rat, acute citalopram treatment significantly reduced 5-HT synthesis in the DRN and MRN (Table 2). In contrast to significant decreases in the cell bodies, the synthesis was significantly increased in several terminal areas (AC, AONM, GP, clustrum, and Cincx, SMCx and FCx). It is important to note that increases in the SPD rats were in some of the limbic structures (*e.g.*, Cincx, AC), which could indicate the brain regions in which antidepressants would be expected to act on the serotonergic system. Similarly, in a human study in depressed patients, SSRIs produced a modulation in the regional glucose utilization in several limbic structures, including the cingulate [22]. Citalopram treatment in depressed humans also showed an effect on regional 5-HT synthesis [3], however those comparisons were not performed following acute administration. Citalopram, as one of the most selective SSRIs, elevates extracellular 5-HT serotonergic cell body and terminal regions [5,17], which in turn reduces intracellular 5-HT stores. A reduction in the intracellular 5-HT stores, especially those in the releasable pool, could upregulate TPH in an attempt to replenish these stores [19]. Sertraline increased TPH mRNA expression and TPH activity (5-HT synthesis) in correspondence with a decrease in intracellular 5-HT stores in the DRN of the SPD rats, as well as in cultured RBL-2H3 cells [19]. The hypothesis related to a need to replenish the neuronal releasable pool is also supported by a reported increase in the extracellular fluorescence and without a change in the intracellular fluorescence for 5-HT after fluoxetine treatment [9], the results of which can be observed only if 5-HT synthesis has been increased to replenish the depleted extracellular pool. Similarly, other drugs that release and/or reduce intracellular 5-HT stores such as reserpine, 3,4-methylenedioxymethamphetamine and fenfluramine, produce increases in 5-HT synthesis at the terminal areas [16,24,29,56]. Further, fluoxetine increased 5-HT synthesis at several brain areas in conscious rats [25,48]. In contrast to the increases in some terminal areas, there were decreases in 5-HT synthesis in the cell body areas (DRN and MRN) of the SPD-CTP rats when these are compared to the SPD-SAL rats. This decrease in the cell bodies is most likely a result of the action of elevated levels of extracellular 5-HT [17,23] producing a downregulation of the synthesis, as previously observed by fluoxetine [25], paroxetine [56], and the 5-HT_{1A} agonists buspirone [32] and flesinoxan [47], in normal rats.

A comparison of 5-HT synthesis between the FSL-CTP and FSL-SAL rats indicates that acute citalopram treatment produces a global reduction in 5-HT synthesis (see Results), with a significant reduction in all of the brain areas investigated (Table 2). This effect is qualitatively and quantitatively different from that in the SPD rats (see above). This overall reduction in synthesis suggests that receptor modulation of 5-HT synthesis is probably very sensitive to the extracellular levels of 5-HT, elevated by citalopram, and/or the intracellular signaling

controlling the activity of TPH is more sensitive to inputs from the membrane receptors. These elevated levels of 5-HT, following citalopram treatment, would, through the activation of the 5-HT_{1A} and/or 5-HT_{1B} autoreceptors, and possibly intracellular cascades, reduce synthesis, to a greater extent, in FSL rats than in the control SPD rats. A similar action of 5-HT_{1A} and 5-HT_{1B} agonists on the modulation of 5-HT synthesis in the terminal areas has been reported [13,32,47].

The above outlined hypothesis in relation to the decreases observed in the FSL rats would also accord with 5-HT_{1A} receptor supersensitivity described to be present in the FSL rats [35,44]. This supersensitivity of the 5-HT_{1A} receptors in the FSL rats has also been shown in a greater hypothermic response to 5-HT_{1A} receptor agonists (8-OH-DPAT and buspirone) than in the FRL [34,35] or SPD rats [44]. In addition to the hypothermic response, the FSL rats displayed behavioral supersensitivity in response to 8-OHDPAT, when compared to the FRL [43] or SPD [44] rats.

5-HT₂ receptor supersensitivity in the FSL rats may be another possible cause to explain the decreased 5-HT synthesis in the FSL rats (Table 2), because 5-HT₂ receptor agonists could induce hyperthermic responses in normal rats [10] and significant behavioral supersensitivities in FSL rats by quipazine [43]. This has also been confirmed by the induction of greater hypothermic effects in FSL rats than in FRL rats [52], with the 5-HT₂ receptor antagonist cyproheptadine. Furthermore, Dremencov et al. [8] observed 5HT_{2C} receptor supersensitivities in the nucleus accumbens of FSL rats, compared to the SPD rats, but they have not performed measurements in FRL rats.

In contrast to the reduction throughout the brain in the FSL rats, the FRL-CTP rats, when compared to the FRL-SAL rats, showed significant increases in 5-HT synthesis in both the cell body areas, and most of the terminal areas (Table 2). These results suggest a somewhat different synthesis control in FRL rats than the normal SPD rats (used as control animals) or FSL rats. This observation would be in line with a subsensitivity of the 5-HT_{1A} receptors observed in the FRL rats relative to those in the FSL rats [34]. This would indicate that the serotonergic feedback system (which could be a receptor itself and/or intracellular signaling) in the FRL rats is less sensitive to the elevation of 5-HT, which is presumably present following citalopram treatment. However, this needs to be confirmed in FRL rats. A depletion of the 5-HT releasable pool would result in the elevation of 5-HT synthesis in an attempt by the system to replenish this pool, as discussed above. Unfortunately, there is no information on tissue concentrations of 5-HT and 5-HIAA in FRL rats, or the effect of antidepressants thereon, and little is known about the differences between the FRL and SPD rats (note that the FRL rats are bred out of SPD rats, but both have been used in different studies as controls). Further studies are needed to obtain a better understanding of the mechanisms by which 5-HT synthesis is controlled in FSL, FRL, and SPD rats. An understanding of these seemingly paradoxical results in the FRL rats could help us understand the ways in which 5-HT synthesis is modulated, and possibly how SSRIs modulate one of the most important parts of serotonergic neurotransmission. However, this observation, along with others that describe the extensive and complex biochemical differences between FRL and SPD rats [35] suggests that it is probably not appropriate to use FRL rats as control animals for studies in FSL rats.

Considering all these results, the negative feedback system of 5-HT neurotransmission in the FSL rats appears to overreact to acute citalopram treatment and the response resembles that in normal rats treated with a 5-HT_{1A} agonist [32]. The abnormal feedback response, compared to that in the SPD controls, to acute citalopram treatment observed in the FSL rats may, at least in part, be related to the response of the serotonergic system in subjects with depressive symptoms, both in this animal model and some humans. As such, it could represent part of the manifestation of depression. A creation of nonphysiological circuitry could also be a result of

the ability of neurons to release, non-synaptically, the neurotransmitter and/or the ability of the noradrenergic neurons to take up 5-HT [20,50]. Both of these actions could be different in the diseased state. This could then contribute to behavioral differences between these strains of rats [35,39,55], and could possibly alter their serotonergic neurotransmission (*e.g.*, affecting synthesis, release, uptake) in FSL rats differently than in FRL or SPD rats.

Conclusion

The effects of acute citalopram treatment on regional 5-HT synthesis rates among FSL, FRL and SPD rats demonstrated that these three strains respond rather differently to citalopram treatment. Only the FSL rats treated acutely with citalopram showed lower 5-HT synthesis rates than their control group in all of the limbic areas, the basal ganglia, and most of the neocortices. If one considers the present data together with other reports on alterations in serotonergic neurotransmission in FSL rats, it can be suggested that an abnormal upregulation of the serotonergic feedback systems could be present in FSL rats. This may, at least in part, contribute to the pathophysiological basis of the FSL rats, a genetic rat model of depression. The data also suggest a lack of synthesis control in the FRL rats, suggesting a breakdown in signal transduction from the extracellular 5-HT levels, assuming elevation by citalopram, and activity of TPH. This could be of interest in the study of autoreceptor desensitization, because acutely, the synthesis is most likely controlled through autoreceptors. The results observed in FRL rats are difficult to explain by the action of an indirect agonist (citalopram), but a deficiency in the intracellular signaling seems to be a reasonable hypothesis. The observations in the FRL and SPD rats point to a difficulty in using FRL rats as controls in studies of FSL rats.

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Abbreviations

α-MTrp	α -[¹⁴ C] methyl-L-tryptophan
Trp	tryptophan
FSL	Flinders Sensitive Line
FRL	Flinders Resistant Line
SPD	Sprague-Dawley
DRN	dorsal raphe nucleus
MRN	median raphe nucleus
TRH	tryptophan hydroxylase
5-HT	5-hydroxytryptamine
5-HIAA	5-hydroxyindoleacetic acid

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Figure 1.

Representative autoradiograms obtained in rats acutely treated with citalopram (CTP; 10 mg/kg) and the control rats treated with the same volume of saline. The autoradiograms shown here are from the rat brains 150 min following the tracer injection, and are presented in pairs (control-treated) for the FSL, SPD and FRL rats. The brain slices were contacted with X-ray films for 3 weeks. The meaning of the structure abbreviations are: DR = raphe dorsal; MR = median; VCx = cortex visual; PCx = cortex parietal; SMCx = cortex sensory-motor; CL = caudate lateral; CM = caudate medial; VT = thalamus ventral; DT = thalamus dorsal; DHi = hippocampus dorsal; VHi = hippocampus ventral; SNc = substantia nigra compacta; SBr substantia nigra reticulata; AC = nucleus accumbens; SC=superior colliculus.

Table 1

Physiological parameters of FSL, SPD and FRL rats treated acutely with citalopram (10 mg/kg) and controls treated by the same volume of saline. The values are given as the mean $\pm$ SD (standard deviation). The number of rats in each group is given in parenthesis below the group identification.

Parameters	FSL		SPD		FRL	
	Control (n=19)	Treated (n=12)	Control (n=14)	Treated (n=17)	Control (n=11)	Treated (n=14)
Body weight (g)	206 $\pm$ 5.5	211 $\pm$ 6.3	215 $\pm$ 6.6	213 $\pm$ 7.3	213 $\pm$ 7.9	205 $\pm$ 5.5
pH	7.41 $\pm$ 0.03	7.40 $\pm$ 0.03	7.41 $\pm$ 0.03	7.39 $\pm$ 0.03	7.41 $\pm$ 0.04	7.41 $\pm$ 0.03
PaO ₂ (mm Hg)	94.9 $\pm$ 8.4	92.2 $\pm$ 8.9	98.5 $\pm$ 7.7	99.2 $\pm$ 14.1	91.2 $\pm$ 9.3	88.4 $\pm$ 8.2
PaCO ₂ (mm Hg)	37.9 $\pm$ 3.4	39.6 $\pm$ 3.2	36.6 $\pm$ 3.1	39.3 $\pm$ 7.7	36.8 $\pm$ 4.2	38.4 $\pm$ 4.2
Plasma total Trp (nmol/mL)	53.5 $\pm$ 11.4	57.8 $\pm$ 10.9	72.2 $\pm$ 15.7*	72.7 $\pm$ 14.2*	53.2 $\pm$ 15.2	50.0 $\pm$ 13.1
Plasma free Trp (nmol/mL)	7.7 $\pm$ 2.5	7.9 $\pm$ 2.4	10.6 $\pm$ 3.6*	11.7 $\pm$ 3.0*	6.6 $\pm$ 1.8	7.4 $\pm$ 2.0

* Significantly different (p<0.05) from the values in FSL and FRL rats

5-HT synthesis rates obtained in FSL, SPD and FRL rats treated acutely with citalopram (10 mg/kg) and controls treated by the same volume of saline. The values are given as the mean±SD (standard deviation). The number of rats in each group is given in parenthesis below the group identification¹

Table 2

Structure	FSL; R (pmol/g/min)			SPD; R (pmol/g/min)			FRL; R (pmol/g/min)		
	Control (n=19)	Treated (n=19)	Difference (%)	Control (n=14)	Treated (n=14)	Difference (%)	Control (n=11)	Treated (n=14)	Difference (%)
Dorsal raphe nucleus	131±17	98±14	-26*	217±34	180±15	-17 [¶]	115±20	134±15	17 [‡]
Median raphe nucleus	107±17	74±14	-30*	173±29	142±15	-18 [¶]	89±16	108±14	22 [‡]
Pontine raphe nucleus	64±9	40±10	-38*	81±13	75±9	ns	43±10	61±10	43 [‡]
Locus coeruleus	45±10	23±11	-47*	48±13	42±9	ns	28±11	45±8	61 [‡]
Substantia nigra reticulata	37±8	20±9	-45*	39±6	42±8	ns	22±8	35±7	59 [‡]
Substantia nigra compacta	43±10	26±9	-41*	48±8	46±7	ns	25±9	46±8	82 [‡]
Ventral tegmental area	47±9	27±9	-42*	53±9	54±7	ns	32±9	53±7	65 [‡]
Globus pallidus	46±9	28±9	-39*	52±6	58±7	10 [¶]	25±7	43±7	72 [‡]
Clastrum	52±10	31±9	-39*	56±7	63±6	12 [¶]	30±9	54±8	78 [‡]
Septal Nucleus	55±9	33±8	-41*	55±7	60±7	ns	27±8	46±73	71 [‡]
Caudate (medial)	64±10	41±10	-37*	70±10	69±8	ns	41±10	65±8	56 [‡]
Caudate (lateral)	50±9	31±9	-38*	54±7	57±7	ns	31±8	51±8	67 [‡]
Thalamus (dorsal)	42±9	23±9	-46*	50±6	49±6	ns	21±7	46±8	115 [‡]
Thalamus (ventral)	43±9	27±9	-38*	43±8	46±6	ns	20±6	45±8	125 [‡]
Hippocampus (dorsal)	52±8	35±10	-33*	72±7	76±5	ns	27±8	59±10	118 [‡]
Hippocampus (ventral)	60±8	42±10	-30*	77±9	80±7	ns	39±10	68±10	75 [‡]
Amygdala	50±9	37±10	-25*	70±7	73±7	ns	25±8	53±7	115 [‡]
Medial geniculate body	47±9	27±9	-42*	45±8	45±8	ns	28±9	51±8	83 [‡]
Lateral geniculate body	49±9	27±9	-44*	47±8	53±7	12 [¶]	25±8	47±8	85 [‡]
Superior chiasmatic nucleus	62±10	41±9	-35*	64±11	71±12	ns	38±10	62±8	63 [‡]
Hypothalamus	55±10	32±10	-42*	63±9	64±9	ns	33±11	63±7	88 [‡]
Accumbens nucleus	57±10	41±9	-27*	72±10	79±7	10 [¶]	39±9	67±9	71 [‡]
Anterior olfactory nucleus (Medial)	47±9	36±8	-23*	57±10	76±7	34 [¶]	24±9	49±8	104 [‡]
Medial forebrain bundle	49±10	27±9	-44*	46±10	45±7	ns	32±9	47±8	ns
Cingulate cortex	44±8	31±9	-29*	50±8	60±5	21 [¶]	23±6	46±10	98 [‡]
Auditory cortex	44±7	27±9	-38*	52±8	54±7	ns	26±8	43±8	65 [‡]
Visual cortex	44±8	29±10	-33*	53±10	57±7	ns	27±10	48±8	80 [‡]
Parietal cortex	41±9	25±8	-38*	45±8	48±6	ns	24±7	45±8	88 [‡]
Sensory-motor cortex	43±9	24±8	-43*	40±7	53±6	30 [¶]	24±9	42±8	75 [‡]
Frontal cortex	40±9	27±9	-33*	42±8	55±5	31 [¶]	23±8	43±8	87 [‡]

¹ ns stands for not significant.

* There is a significant difference between the synthesis in the saline and citalopram treated FSL rats.

[¶] Identifies a significant difference between the synthesis in the saline and citalopram treated SPD rats.

[‡] There is a significant difference between the synthesis in the saline and citalopram treated FRL rats.