ELSEVIER

European Journal of Pharmacology 352 (1998) 307–315

ejp

Long-term effects on serotonin transporter mRNA expression of chronic neonatal exposure to a serotonin reuptake inhibitor

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Received 4 September 1997; revised 24 April 1998; accepted 30 April 1998

Abstract

Chronic administration of clomipramine or other serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibitors to neonatal rats produces behaviours that resemble a depressive state in the adult animal, and this model is therefore regarded as a putative animal model of depression. Alterations in the activity of the central 5-HT system are important in understanding the pathophysiology of depression, and therefore, we examined whether this model was associated with changes in the expression of 5-HT_{1A} receptor, 5-HT_{1B} receptor, and 5-HT transporter mRNA in the dorsal raphe nucleus and the hippocampus. Wistar rats were injected twice daily with the serotonin reuptake inhibitors clomipramine and 5-chloro-1-[3-(dimethylamino)propyl]-1-(4-fluoro-phenyl)-1,3-dihydroiso-benzofurane, hydrochloride (code Lu 10-134-C) at doses of 15 mg kg^{-1} or vehicle i.p. from postnatal day 8 for 14 days. Groups of rats ($n = 10$) were either killed the day after the last injection or left undisturbed for 69 days before they were killed. The expression of 5-HT transporter, 5-HT_{1A} receptor, and 5-HT_{1B} receptor mRNA was examined in the dorsal raphe nucleus and in the CA1 of the hippocampus by means of quantitative in situ hybridisation histochemistry. Both compounds resulted in an increase in 5-HT transporter mRNA expression (40% more than vehicle) in the dorsal raphe nucleus the day after the last injection (postnatal day 22). A small but significant increase in 5-HT_{1B} receptor mRNA expression in the CA1 was seen after clomipramine, but not after Lu 10-134-C, probably reflecting clomipramine's affinity for both the 5-HT and noradrenaline transporters as well as for a number of monoamine receptor sites. Levels of 5-HT_{1A} receptor mRNA were unchanged. In contrast, 5-HT transporter mRNA expression in the dorsal raphe nucleus was significantly decreased in the adult after neonatal treatment with either of the two drugs compared to vehicle. No changes in 5-HT_{1A} receptor and 5-HT_{1B} receptor mRNA expression were observed in any of the regions examined in these animals. The results show that the persistent depressive behaviour previously shown in this model is also associated with changes in the expression of 5-HT transporter mRNA. This long-term alteration in gene expression may result from disturbances in 5-HT neurotransmission in the brain of the neonatal animals.

Keywords: 5-HT (5-hydroxytryptamine, serotonin); Reuptake; Transporter mRNA; Raphe nuclei; Antidepressant

1. Introduction

The serotonin (5-hydroxytryptamine; 5-HT) transporter (5-HT transporter) plays a key role in the termination of 5-HT neurotransmission by sodium-dependent uptake of 5-HT into the presynaptic terminal. Selective serotonin reuptake inhibitors such as citalopram, fluoxetine, and paroxetine are widely used in the treatment of depression, anxiety disorders, obsessive-compulsive disorder, eating disorders, and substance abuse including alcoholism (Murphy, 1990).

Neonatal exposure of rats to the tricyclic antidepressant clomipramine during the second and third postnatal weeks induces, in the adult rat, behavioural and physiological changes that resemble endogenous depression in humans (Vogel et al., 1990a). The most consistent behavioural changes in the animals include locomotor hyperactivity in the open-field test (Hartley et al., 1990; Mirmiran et al., 1981), diminished pleasure-seeking behaviour (Vogel et al., 1990a,b), reduced aggressiveness (Vogel et al., 1988),

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0014-2999/98/$19.00

and increased immobility time ('behavioural despair') in the forced swim test (Fernandez-Pardal and Hilakivi, 1989; Hansen et al., 1997; Velazquez-Moctezuma and Ruiz, 1992).

Neurochemically, the neonatal treatment attenuates firing frequency in the dorsal raphe nucleus and increases 5-HT metabolism in the brain stem of the adult rat (Hilakivi et al., 1995; Yavari et al., 1993). It is generally considered that a deficit in 5-HT neurotransmission is linked to the pathophysiology of depression (Charney et al., 1990; Delgado et al., 1994; Grahame-Smith, 1989; Meltzer and Lowry, 1987), and the 5-HT transporter gene is considered a candidate locus for polymorphism in anxiety and affective disorders (Collier et al., 1996; Lesch et al., 1996). The aim of the present study was to explore whether the depressive state induced in this model was associated with changes in the expression of genes important in depressed 5-HT neurotransmission. The objective was to compare the observed changes to those seen in depressed patients. In particular, we were, in the first place, interested to examine if the behavioural alterations seen after neonatal administration of Lu 10-134-C and clomipramine (Hansen et al., 1997) could be found to correlate to changes in 5-HT transporter, 5-HT_{1A} receptor, or 5-HT_{1B} receptor gene expression.

Most behavioural analyses reported in recent years were carried out with clomipramine, but this compound has affinity for both the 5-HT and the noradrenaline transporters, as well as for a number of central receptor sites (Hyttel, 1994). We therefore compared the effects of clomipramine with those of Lu 10-134-C, which exhibits selectivity for the 5-HT reuptake site (Bigler et al., 1977; Hansen et al., 1997), by means of semi-quantitative in situ hybridisation histochemistry. This method was chosen because provides a detailed anatomical resolution of mRNA transcription. 5-HT is important in the regulation of a wide variety of cortical functions and has also been implicated in the modulation of appetite, memory, mood, emotionality, thermoregulation, and sexual behaviour (Jacobs and Azmitia, 1992). Furthermore, 5-HT receptors are widely distributed in the brain, with the highest levels of 5-HT_{1A} receptor and 5-HT_{1B} receptor mRNA being observed in the hippocampus (Bruinvels et al., 1994; Wright et al., 1995). Since the hippocampus is also suggested to be a key component in the mediation of depression (Duman et al., 1997), the expression of 5-HT_{1A} receptor and 5-HT_{1B} receptor mRNA was measured in this structure. The level of 5-HT transporter mRNA has been shown to be influenced by selective serotonin reuptake inhibitors in the dorsal raphe nucleus (Lesch et al., 1993), and we therefore also examined the effects of selective serotonin reuptake inhibitors in the neonatal and adult animal in this structure as well. Together, these estimates should provide an indirect correlate of 5-HT neurotransmission in the neonate and adult rat after treatment with selective serotonin reuptake inhibitors.

2. Materials and methods

2.1. Animals and treatment

Wistar rats were bred at H. Lundbeck A/S from stock purchased from Charles River. The male neonates were divided into three groups ($n = 10$), each of which consisted of pups from 2 or 3 litters. They received an i.p. dose of 15 mg kg^{-1} Lu 10-134-C (5-chloro-1-[3-(dimethyl amino)propyl]-1-(4-fluorophenyl)-1,3-dihydroisobenzofurane, hydrochloride), or 15 mg kg^{-1} clomipramine hydrochloride dissolved in sterile isotonic saline, or an equal volume (5 ml kg^{-1}) of isotonic saline twice daily for 14 consecutive days from postnatal day 8 to 21 (P8–P21). The drugs were synthesised by the Department of Medical Chemistry, H. Lundbeck A/S, Copenhagen-Valby, Denmark. During treatment, the rats were weighed once daily. Twenty-four hours after the last injection (P22) the first group of pups ($n = 10$ in each group) treated with drugs or vehicle were decapitated and brains were collected and frozen at −80°C until use. A similar number of rats were removed from their dams and kept under a 12-h light/dark cycle in Macron type III cages (3 rats per cage). The rats had free access to standard food and tap water and were weighed weekly. Room temperature (21 ± 2°C), relative humidity (55 ± 5%), and air exchange (16 times per h) were automatically controlled. At P91 these rats ($n = 10$) were decapitated and brains were collected. After removal, the brains were frozen on dry ice and stored at −80°C until sectioning. The tissue was sectioned in coronal 12-μm thick serial sections on a cryostat and sections were thaw-mounted onto 5 gelatine-coated glass slides. The same area of the dorsal raphe nucleus between brains was defined as the region where the decussation of the superior cerebellar peduncle was observed.

2.2. In situ hybridisation histochemistry

Sections on one glass slide per animal were hybridised for a specific mRNA. The in situ hybridisation histochemical procedure was carried out as previously described (Larsen et al., 1993). Sections were prepared in 4% formaldehyde and rinsed twice in phosphate-buffered saline (PBS). Subsequently, the slides were treated with 0.25% acetic anhydride in 0.1 M triethanolamine/0.9% NaCl. Furthermore, sections were delipidated in a series of graded ethanol solutions (70, 80, 95 and 100%) for 2 min each, followed by treatment in 100% chloroform for 5 min. They were finally rinsed briefly in 95% and 100% ethanol. Sections were allowed to dry at room temperature before the in situ hybridisation histochemistry procedure was started.

The selected probes, as shown in Table 1, were labelled at their 3′ end using [α-^{35}S]thio-dATP (1000 Ci mmol^{-1}, Amersham) and terminal deoxynucleotidyl transferase (TdT, Boehringer Mannheim, Denmark) to obtain a spe-

Table 1
Synthetic oligonucleotides

Probe	Sequence (5′–3′)	Base no.	Reference
5-HT transporter mRNA	ACTGCAGAGTACCCATTGGATATTTGGCTAGGCTCTGCCCTGTCCGCTGT	77–126	Fujita et al., 1993;
5-HT_{1A} mRNA	ACGAAGTTCCTAAGCTGGTGCCTGCTCCCTTCTTTTCCACCTTCCTGAC	810–858	Hoffman et al., 1991
	GCCTCACTGCCCCATTAGTGCACGGAGTCCCCACCGCCCTGTTCTCA	923–969	Albert et al., 1990
5-HT_{1B} mRNA	TGATGGCCGCCCTTTTGGGAGTTCTTTTAGCAGAATAGTCCACCGCAT	745–790	
	GAAATCGAGATGGAGAAGACCCACACCAGCACGATCATGATGGCCGC	923–969	Bruinvels et al., 1994; Spurlock et al., 1994

Sequences are listed from 5′ to 3′ and are complimentary to the base number listed. The oligonucleotides were synthesised by using an Applied Biosystems model A DNA synthesizer.

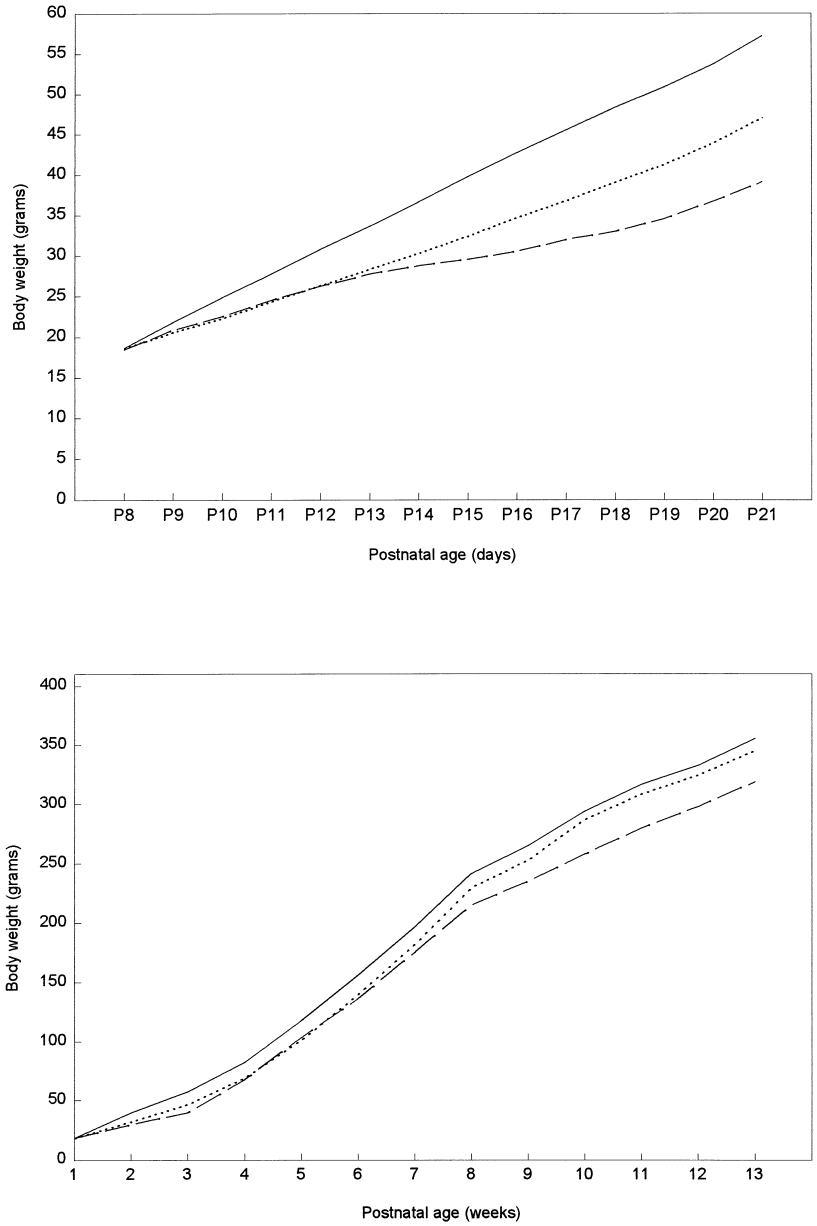

Fig. 1. This figure shows the weight of the experimental rats during (upper figure) and after treatment (lower figure). Lu 10-134-C (dotted line), clomipramine (dashed line) and vehicle (filled line). As can be seen, both compounds significantly affected postnatal weight gain, whereas only Lu 10-134-C decreased weight gain significantly in the adult animal.

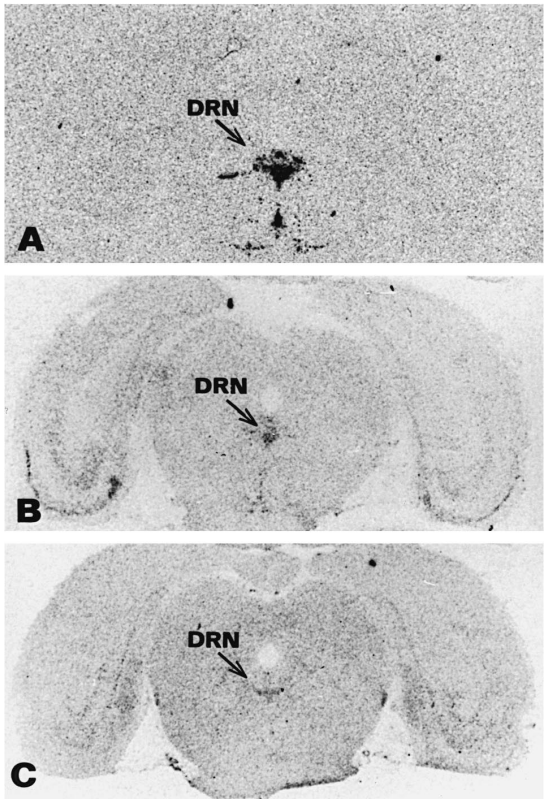

Fig. 2. Photographs of autoradiograms of frontal section through the dorsal raphe nucleus (DRN) at the level of the crossover of the cerebellar peduncle hybridised for 5-HT transporter receptor mRNA (A), 5-HT_{1A} receptor mRNA (B), and 5-HT_{1B} receptor mRNA (C). The sections were taken from a control rat killed at postnatal day 22. The expression of 5-HT transporter mRNA is very strong and present in both the dorsal and the median raphe nucleus and the scattered population of cells within the B9 region. Similarly, the expression of 5-HT_{1A} receptor and 5-HT_{1B} receptor mRNA can be detected in the dorsal raphe nucleus.

cific activity of approximately 2.0×10^{19} dpm mol^{-1}. For all probes, a specific activity of 1.4×10^{7} dpm ml^{-1} (10^{7} cpm ml^{-1}) was added to a buffer solution (pH 7.2) containing 50% (v/v) formamide, 4 × standard saline citrate buffer, 1 × Denhardts solution, 10% dextran sulphate (w/v), 0.5 mg ml^{-1} salmon sperm DNA, 0.25 mg ml^{-1} yeast tRNA and 10 mM dithiotreitol. When a mixture of two different anti-sense probes complimentary to the same mRNA were used, they contributed equally by 50:50 of the total specific activity.

After incubation at 37°C in a humid chamber for 22 h, sections were washed in 1 × standard saline citrate buffer at 55°C for 4 × 15 min in 1 × standard saline citrate buffer, and at room temperature for 2 × 30 min. Excess salt was removed with demineralised water and brain sections were blown dry and exposed on X-ray films (Hyperfilm β-Max, Amersham). The films were exposed to slides with dorsal raphe nucleus for 3 days for 5-HT transporter mRNA and 14 days for 5-HT_{1A} receptor and 5-HT_{1B} receptor mRNA levels. Films exposed to hippocampal sections were stored at 4°C for 21 days.

The specificity of the probes used in this study was confirmed by using at least two oligonucleotide probes to compare their equal distribution patterns. The entire experiment was repeated twice with similar results.

2.3. Measurements and statistics

Developed film autoradiograms were quantified by using an image analysis system (MCID M1 v.4.2 r.2.0, Imaging Research, Canada). Optical grain density was measured interactively, using 3.3 Bq and 74 Bq precalibrated ^{14}C microscale standards (Amersham, UK) to ^{35}S tissue equivalents as reference. The densitometric data were corrected for non-specific signals collected from the central tegmental tract (for dorsal raphe nucleus densitometry) and corpus callosum (for subhippocampal densitometry). Densitometric data collected from three sections per animal were calculated as means. Even though some animals were excluded during the histological procedures, the number of animals exceeded eight in all experiments. Statistical evaluation was carried out by a one-way analysis of variance (ANOVA) followed by Newman–Keul's post hoc test, using a statistical software program (Crunch v.4.0). A *P*-value less than 0.05 was considered significant.

3. Results

Because the two compounds used are anorectic agents (Leonard, 1994), the weight gains during the period of

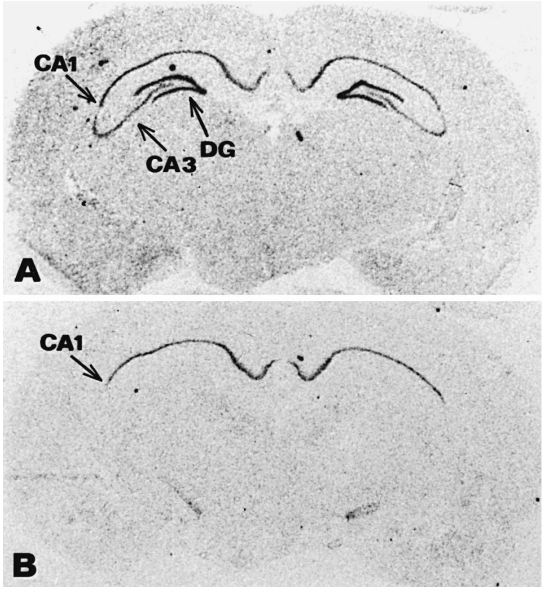

Fig. 3. Photographs of autoradiograms illustrating the expression of 5-HT_{1A} receptor and 5-HT_{1B} receptor mRNA in the hippocampus. The expression of 5-HT_{1A} receptor mRNA is strong in both the CA1 and CA3 regions of the hippocampus, as well as in the dentate gyrus (DG), whereas expression of 5-HT_{1B} receptor mRNA is observed exclusively in the CA1 region.

treatment and the subsequent period without treatment are shown in Fig. 1. The animals displayed different weight curves: rats given Lu 10-134-C had the lowest weight gain. At the end of the treatment period, the three groups of rats differed significantly ($P < 0.05$) in weight (Fig. 1), and about the same percentage weight difference between the groups was maintained until the adult rats were killed.

3.1. Expression of 5-HT transporter, 5-HT$_{1A}$ receptor, and 5-HT$_{1B}$ receptor mRNA in the raphe nuclei and hippocampus

The rat brain sections hybridised for two different oligonucleotides complimentary to the same mRNA revealed an identical pattern of labelling in the raphe nuclei and other brain regions. The expression of 5-HT transporter mRNA in the raphe nuclei was very strong (Fig. 2A). Distinct clusters of labelling in the dorsal raphe subnuclei, the median raphe nucleus, and the B9 located in the ventral pons could be detected (Fig. 2A). Because of the small size of the median raphe nucleus at this level, this area was not analysed. Levels of 5-HT transporter mRNA were undetectable in the surrounding grey matter of the pons and in the cerebral cortex. Expression of 5-HT$_{1A}$ receptor and 5-HT$_{1B}$ receptor mRNA was concentrated in the dorsal raphe nucleus (Fig. 2B,C), but the level in the central region of the dorsal raphe nucleus appeared more dense than in the lateral wings and in the median

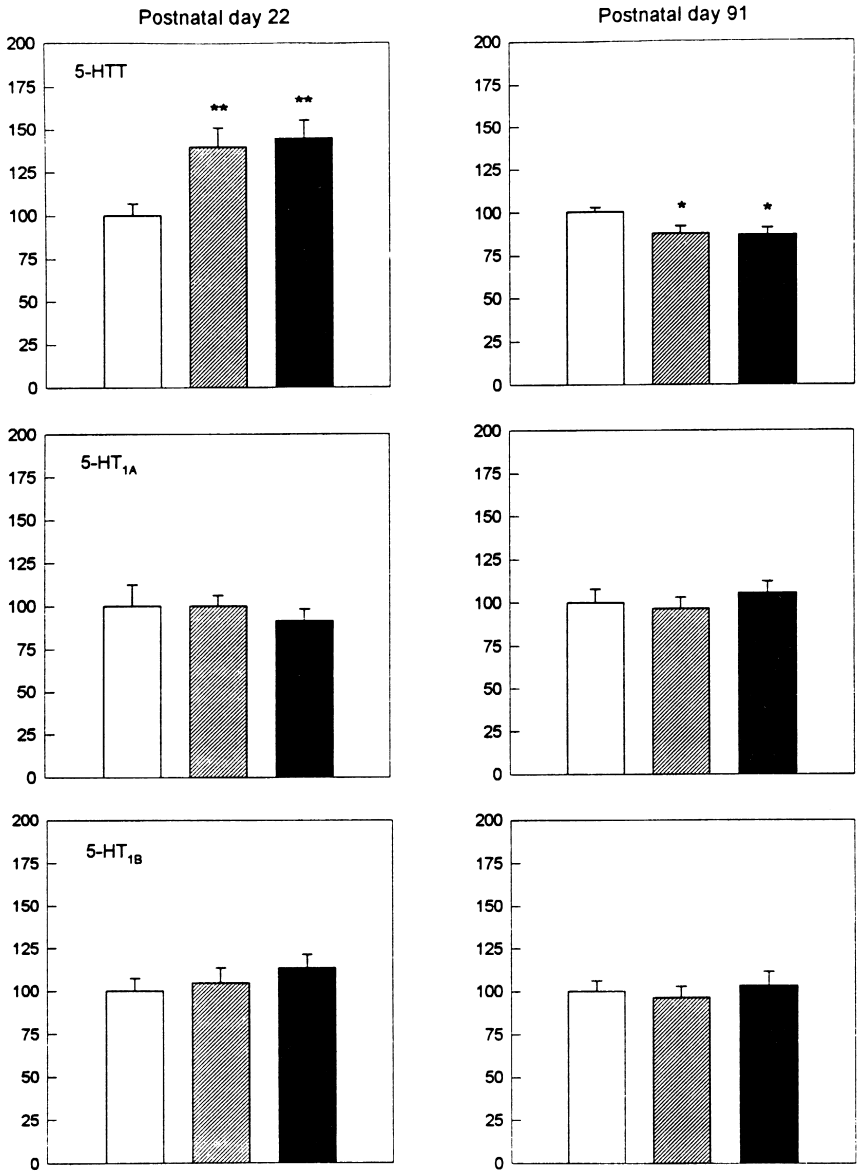

Fig. 4. Quantitative analysis of the level of 5-HT transporter mRNA, 5-HT$_{1A}$ receptor mRNA, and 5-HT$_{1B}$ receptor mRNA levels in the dorsal raphe nucleus of rats killed either at postnatal day 22 (left panel) or at postnatal day 91 (right panel). The animals were either treated with vehicle (open bars), 15 mg kg^{-1} Lu 10-134-C (hatched bars) or clomipramine (filled bars). The ordinate is the concentration of mRNA, where the expression measured in animals treated with vehicle is defined as 100. $**P < 0.001$; $*P < 0.05$.

raphe nucleus, probably reflecting the densest concentration of 5-HT-producing cell bodies in this region. In the same section, 5-HT_{1A} receptor and 5-HT_{1B} receptor mRNA expression was observed in the inferior colliculus and in the cerebral cortex (Fig. 2B,C). A particularly strong expression of 5-HT_{1A} receptor mRNA was observed in the pyramidal layer and the fascia dentata of the hippocampal formation (Fig. 3A). Dense 5-HT_{1B} receptor mRNA expression was detected in the CA1 region as well as in the subthalamic nucleus, whereas other parts of the hippocampus contained low or undetectable levels of labelling (Fig. 3B).

3.2. Effects of neonatal administration with selective serotonin reuptake inhibitors in neonatal rats

A significant increase in the expression of 5-HT transporter mRNA was detected in the dorsal raphe nucleus of neonatal rats shortly after a 14-day exposure to either clomipramine or Lu 10-134-C (Fig. 4). The two drugs produced about the same increase in the mRNA expression. In contrast, the levels of 5-HT_{1A} receptor and 5-HT_{1B} receptor mRNAs in the dorsal raphe nucleus were not affected in these animals. In the hippocampus, a significant increase of about 15% in the density of 5-HT_{1B} receptor mRNA was detected after clomipramine, but not Lu 10-134-C (Fig. 5). No change was detected in the amount of 5-HT_{1A} receptor mRNA in the CA1 (Fig. 5) or in other hippocampal areas such as CA3 and fascia dentata (not shown).

3.3. Effects of neonatal administration with selective serotonin reuptake inhibitors in adult rats

In adult rats treated neonatally with clomipramine or Lu 10-134-C, the level of 5-HT transporter mRNA in the dorsal raphe nucleus was significantly ($P < 0.05$) decreased compared to that of vehicle-treated rats (Fig. 4). No change of dorsal raphe or hippocampal 5-HT_{1A} receptor and 5-HT_{1B} receptor mRNA content was detected (Figs. 4 and 5).

4. Discussion

4.1. Effects of neonatal treatment in the rat

Semi-quantitative in situ hybridisation histochemistry demonstrated a significant increase in the expression of 5-HT transporter mRNA in the dorsal raphe nucleus after

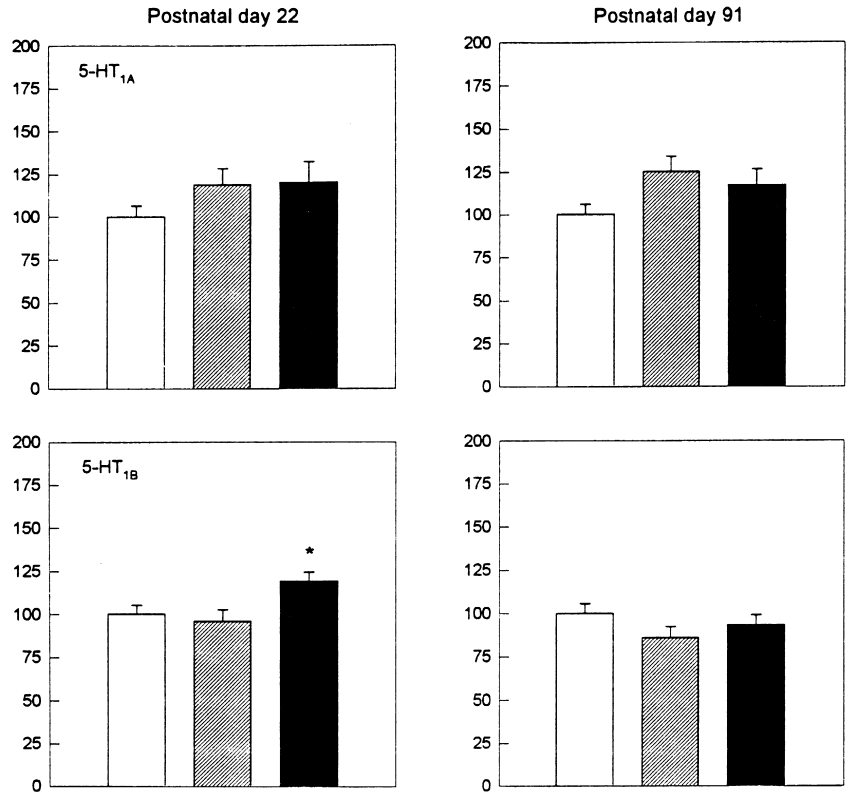

Fig. 5. Quantitative analysis of the level of 5-HT_{1A} (upper row) and 5-HT_{1B} (lower row) mRNA levels in the CA1 region of the hippocampus of rats killed either at postnatal day 22 (left panel) or at postnatal day 91 (right panel). The animals were either treated with vehicle (open bars), 15 mg kg^{-1} Lu 10-134-C (hatched bars) or clomipramine (filled bars). The ordinate is the concentration of mRNA, where the expression measured in animals treated with vehicle is defined as 100. $* P < 0.05$.

chronic administration of selective and non-selective 5-HT reuptake inhibitors. Similar studies with adult rats demonstrated that administration of classical antidepressants including selective serotonin reuptake inhibitors either does not change (Spurlock et al., 1994) or increases levels of 5-HT transporter mRNA (Lesch et al., 1993). An obvious explanation for the apparently greater increase in the expression of 5-HT transporter mRNA in the present study is that neonatal rats are more sensitive to selective serotonin reuptake inhibitors than adult rats. The results may also be due to the fact that the animals were treated with different concentrations of the compounds, or because the various compounds may exhibit different rates of metabolism in adult vs. neonatal rats. Furthermore, it should be kept in mind that some studies with adult rats used Northern analysis to detect 5-HT transporter mRNA expression in whole brain or in midbrain homogenates, which may have prevented the detection of regionalised changes in 5-HT transporter expression in the dorsal raphe nucleus.

Several lines of evidence suggest that 5-HT is important in the development of the brain (Pranzatelli, 1994; Pranzatelli and Martens, 1992), and the level of 5-HT transporter mRNA has been found to be related to synaptogenesis (Ivgy-May et al., 1994). Thus, an increase in 5-HT neurotransmission affects the developmental process and results in axonal sprouting of the developing neurons, which again indirectly produces a rise in 5-HT transporter mRNA expression. It is likely that administration of selective serotonin reuptake inhibitors in the neonatal rat stimulates differentiation or a sprouting process through 5-HT receptors in the dorsal raphe nucleus, and that this growth produces the increase in 5-HT transporter mRNA expression. That selective serotonin reuptake inhibitors have direct actions on the raphe neurons is supported by the results of Foguet et al. (1993) showing that differentiation of 5-HT neurons in vitro is affected by activation of protein kinase A via a cAMP-dependent cascade. It is unlikely that neonatal treatment with selective serotonin reuptake inhibitors produces an increased number of perikarya as no increase was seen in 5-HT_{1A} receptor and 5-HT_{1B} receptor mRNA expression in the dorsal raphe nucleus.

An increased expression of 5-HT_{1B} receptor mRNA in the CA1 region of the hippocampus was found in animals treated with clomipramine, but not with a more selective serotonin reuptake inhibitor. Accordingly, the change in 5-HT_{1B} receptor gene expression cannot directly be attributed to 5-HT neurotransmission alone, but may be related to either the affinity of clomipramine for the noradrenaline transporter or for another monoamine receptor.

Autoradiographic studies have shown a reduction in the number of 5-HT_{1A} receptor binding sites in the dorsal raphe nucleus after prolonged treatment with selective serotonin reuptake inhibitors in the adult rat (Welner et al., 1989). Chronic treatment with selective serotonin reuptake inhibitors in the adult rat also enhances the effectiveness of 5-HT synaptic transmission in the rat hippocampus, probably via 5-HT_{1A} receptor autoreceptor desensitisation (Chaput et al., 1991). No evidence for changes in 5-HT_{1A} receptor gene expression was found in the present experiment. This emphasises the difference between neonatal and adult rats or reflects post-transcriptional processing of 5-HT_{1A} receptor molecules.

4.2. Effects of neonatal treatment in the adult rat

It is interesting that neonatal treatment with selective serotonin reuptake inhibitors produced long-term effects on 5-HT transporter mRNA expression. Even though the animals were left undisturbed for 69 days after the treatment, a significant down-regulation of 5-HT transporter mRNA levels was observed. It is tempting to speculate about the relationship between transcription and the behavioural deficits described previously (Fernandez-Pardal and Hilakivi, 1989; Mirmiran et al., 1981; Vogel et al., 1990b). A decreased function of the transporter could compensate for a long-term suppression of 5-HT release. If the augmented concentration of synaptic 5-HT concentrations led to reduced levels of 5-HT transporter mRNA and perhaps to a similar reduction in the synaptic concentration of 5-HT, this could provide support that a decrease in 5-HT levels is involved in the abnormal behaviours seen in the animals.

4.3. Mechanisms linking neonatal exposure to selective serotonin reuptake inhibitors with changes in 5-HT transporter mRNA

Antidepressant-sensitive noradrenaline and serotonin transporters are closely related members of the Na^+/Cl^- transporter gene family, whose other members include transporters for inhibitory amino acid transmitters, neuromodulators, osmolytes and nutrients (Blakely et al., 1991, 1994; Hoffman et al., 1991). The mechanism involved in the regulation of the 5-HT transporter gene in the living brain is unknown. At the cellular level, staurosporine and cholera toxin up-regulate, via a cAMP-independent signalling pathway, 5-HT transport activity in human placental choriocarcinoma cells by increasing the steady state levels of 5-HT transporter mRNA and by the consequent increase in the transporter density in the plasma membrane (Ramamoorthy et al., 1993, 1995).

5-HT transporters and 5-HT autoreceptors are abundantly expressed in the serotonergic dorsal raphe nucleus (Albert et al., 1990; Fujita et al., 1993; Sotelo et al., 1990; Wright et al., 1995), and it is, therefore, likely that 5-HT neurotransmission mediated by autoreceptors on 5-HT neurons in the dorsal raphe nucleus is responsible for the effect. Another possibility is that 5-HT transporter mRNA expression is regulated via a negative feedback loop to the dorsal raphe nucleus, linking the increased 5-HT neurotransmission back to the dorsal raphe nucleus via central

inputs. Several inputs to the dorsal raphe nucleus from the substantia nigra, locus coeruleus, lateral habenula, parabrachial nucleus, and posterior hypothalamus contain transmitters that could potentially affect spontaneous electrical activity and thereby regulate 5-HT transporter gene expression (Lakoski and Aghajanian, 1983; Nishikawa and Scatton, 1985; Stern et al., 1981; Vandermaelen and Aghajanian, 1983).

Yet another possibility is that the state of the animals indirectly affects 5-HT neurotransmission via the hypothalamo–pituitary–adrenal axis. Support for this hypothesis comes from the fact that the animals exhibited an altered weight curve, emphasising the well-known observation that selective serotonin reuptake inhibitors affects homeostasis in animals during development (Curzon et al., 1997). It is known that 5-HT affects neuroendocrine regulation (Jacobs and Azmitia, 1992), and the raphe projects to centres involved in homeostasis (Larsen et al., 1996). It is also well-known that glucocorticoids affect the 5-HT system at many targets in an age-dependent manner (Laaris et al., 1995), and the possibility that glucocorticoids are involved in the described effects can therefore not be excluded.

4.4. Technical, functional, and clinical consideration

Though this study documents major changes in gene expression, any functional consequences are difficult to conclude from the present material alone. The technique of in situ hybridisation histochemistry used in the present study measures only the steady-state levels of mRNA. Neither the rate of synthesis of specific transcripts (transcription rate), nor their stability are reflected by such measurements. Additionally, the applied methods do not document the rate of translation of an mRNA species to functional receptors or transporters. In some cases (Dewar et al., 1992; Linnet et al., 1995), changes in the level of binding at the transporter binding sites do not reflect the level of mRNA expression, indicating that there is a change in the rate of turnover of transporter molecules in cells which is independent of the level in the gene encoding them at a specific time. Further radioligand binding studies are needed to address this point in more detail.

4.5. Conclusion

Several lines of evidence support the notion that a reduction in the central activity of the central 5-HT system is implicated in the pathophysiology of depression (Delgado et al., 1994; Meltzer and Lowry, 1987), and compounds which block 5-HT uptake have been found to be effective antidepressants (Broekkamp et al., 1995; Delgado et al., 1994; Meltzer and Lowry, 1987), presumably due to their capacity to increase 5-HT neurotransmission (Moret and Briley, 1996). It is, therefore, interesting that in the neonatal model present there were both long-term changes in the 5-HT transporter mRNA in the adult rats and behavioural abnormalities. Several studies are now needed to determine whether the mRNA is regulated functionally, and whether this model can be used as an animal model of depression.

Acknowledgements

We thank Tine Ø. Gerlén and Pia M. Carstensen for expert technical assistance, Tina Bott for typing the manuscript and Sandra Hogg and Arne Mørk for their critical reading of the manuscript.

References

Albert, P.R., Zhou, Q.-Y., Van Tol, H.H.M., Bunzow, J.R., Civelli, O., 1990. Cloning, functional expression, and mRNA tissue distribution of the rat 5-hydroxytryptamine$_{1A}$ receptor gene. J. Biol. Chem. 265, 5825–5832.

Bigler, A.J., Bøgesø, K.P., Toft, A., 1977. Quantitative structure-activity relationships in a series of selective 5-HT uptake inhibitors. Eur. J. Med. Chem. 12, 289–295.

Blakely, R.D., Berson, H.E., Fremeau Jr., R.T., Caron, M.G., Peek, M.M., Prince, H., Bradley, C.C., 1991. Cloning and expression of a functional serotonin transporter from rat brain. Nature 354, 66–70.

Blakely, R.D., De, F.L., Hartzell, H.C., 1994. Molecular physiology of norepinephrine and serotonin transporters. J. Exp. Biol. 196, 263–281.

Broekkamp, C.L.E., Leysen, D., Peeters, B.W.M.M., Pinder, R.M., 1995. Prospects for improved antidepressants. J. Med. Chem. 38, 4615–4633.

Bruinvels, A.T., Landwehrmeyer, B., Gustafson, E.L., Durkin, M.M., Mengod, G., Branchek, T.A., Hoyer, D., Palacios, J.M., 1994. Localisation of 5-HT$_{1B}$, 5-HT$_{1D\alpha}$ 5-HT$_{1E}$ and 5-HT$_{1F}$ receptor messenger RNA in rodent and primate brain. Neuropharmacology 33, 367–386.

Chaput, Y., De Montigny, C., Blier, P., 1991. Presynaptic and postsynaptic modifications of the serotonergic system by long-term administration of antidepressant treatments. Neuropsychopharmacology 5, 219–229.

Charney, D.S., Crystal, J.H., Delgado, P.L., Heninger, G.R., 1990. Serotonin-specific drugs for anxiety and depressive disorders. Annu. Rev. Med., pp. 437–446.

Collier, D.A., Stober, G., Li, T., Heils, A., Catalano, M., Di Bella, D., Arranz, M.J., Murray, R.M., Vallada, H.P., Bengel, D., Muller, C.R., Roberts, G.W., Smeraldi, E., Kirov, G., Sham, P., Lesch, K.P., 1996. A novel functional polymorphism within the promoter of the serotonin transporter gene: possible role in susceptibility to affective disorders. Mol. Psychiatry 1, 453–460.

Curzon, G., Gibson, E.L., Oluyomi, A.O., 1997. Appetite suppression by commonly used drugs depends on 5-HT receptors but not on 5-HT availability. Trends Pharmacol. Sci. 18, 21–25.

Delgado, P.L., Price, L.H., Miller, H.L., Salomon, R.M., Aghajanian, G.K., Heninger, R., Charney, D.S., 1994. Serotonin and the neurobiology of depression. Arch. Gen. Psychiatr. 51, 865–874.

Dewar, K.M., Grondin, L., Carli, M., Lima, L., Reader, T.A., 1992. [^{3}H]paroxetine binding and serotonin content of rat cortical areas, hippocampus, neostriatum, ventral mesencephalic tegmentum, and midbrain raphe nuclei region following *p*-chlorophenylalanine and *p*-chloroamphetamine treatment. J. Neurochem. 58, 250–257.

Duman, R.S., Heninger, G.R., Nestler, E.J., 1997. A molecular and cellular theory of depression. Arch. Gen. Psychiatry 54, 597–606.

Fernandez-Pardal, J., Hilakivi, L.A., 1989. Effect of low alcohol dose on behavioral despair in rats neonatally treated with antidepressant drugs. Alcohol 6, 93–95.

Foguet, M., Hartikka, J.A., Schmuck, K., Lübbert, H., 1993. Long-term regulation of serotoninergic activity in the rat brain via activation of protein kinase. EMBO J. 12, 903–910.

Fujita, M., Shimada, S., Maeno, H., Nishimura, T., Tohyama, M., 1993. Cellular localisation of serotonin transporter mRNA in the rat brain. Neurosci. Lett. 162, 59–62.

Grahame-Smith, D.G., 1989. Serotonin function in affective disorders. Acta. Psychol. Scand. 80, 7–12.

Hansen, H., Sanchez, C., Meier, E., 1997. Neonatal administration of the selective serotonin reuptake inhibitor Lu 10-134-C increases forced swimming-induced immobility of adult rats: a putative animal model of depression?. J. Pharm. Exp. Ther. 283, 1333–1341.

Hartley, P., Neill, D., Hagler, M., Kors, D., Vogel, G., 1990. Procedure- and age-dependent hyperactivity in a new animal model of endogenous depression. Neurosci. Biobehav. Rev. 14, 69–72.

Hilakivi, I., Ahtee, L., Rinne, O., Taira, T., Attila, L.M.J., Marjamäki, P., 1995. Effects of monoamine uptake inhibitors given early postnatally on monoamines in the brain stem, caudate/putamen and cortex, and on dopamine D_1 and D_2 receptors in the caudate/putamen. J. Neural. Trans. 102, 139–148.

Hoffman, B.J., Mezey, E., Brownstein, M.J., 1991. Cloning of a serotonin transporter affected by antidepressants. Science 254, 579–580.

Hyttel, J., 1994. Pharmacological characterisation of selective serotonin reuptake inhibitors (SSRIs). Int. Clin. Psychopharmacol. 9, 19–26.

Ivgy-May, N., Tamir, H., Gershon, M.D., 1994. Synaptic properties of serotonergic growth cones in developing rat brain. J. Neurosci. 14, 1101–1129.

Jacobs, B.L., Azmitia, E.C., 1992. Structure and function of the brain serotonin system. Physiol. Rev. 72, 165–229.

Laaris, N., Haj-Dahmane, S., Hamon, M., Lanfumey, L., 1995. Glucocorticoid receptor-mediated inhibition by corticosterone of 5-HT_{1A} autoreceptor functioning in the rat dorsal raphe nucleus. Neuropharmacology 34, 1201–1210.

Lakoski, J.M., Aghajanian, G.K., 1983. Effects of histamine, H1- and H2-receptor antagonists on the activity of serotonergic neurons in the dorsal raphe nucleus. J. Pharmacol. Exp. Ther. 227, 517–523.

Larsen, P.J., Mikkelsen, J.D., Jessop, D.S., Lightman, S.L., Chowdrey, H.S., 1993. Neuropeptide Y mRNA and immunoreactivity in hypothalamic neuroendocrine neurons: effects of adrenalectomy and chronic osmotic stimulation. J. Neurosci. 13, 1138–1147.

Larsen, P.J., Hay-Schmidt, A., Vrang, N., Mikkelsen, J.D., 1996. Origin of projections from the midbrain raphe nuclei to the hypothalamic paraventricular nucleus in the rat: a combined retrograde and anterograde tracing study. Neuroscience 70, 963–988.

Leonard, B.E., 1994. Serotonin receptors—where are they going?. Int. Clin. Psychopharmacol. 9, 7–17.

Lesch, K.P., Aulakh, C.S., Wolozin, B.L., Tolliver, T.J., Hill, J.L., Murphy, D.L., 1993. Regional brain expression of serotonin transporter mRNA and its regulation by reuptake inhibiting antidepressants. Mol. Brain Res. 17, 31–35.

Lesch, K.P., Bengel, D., Heils, A., Sabol, S.Z., Greenberg, B.D., Petri, S., Benjamin, J., Muller, C.R., Hamer, D.H., Murphy, D.L., 1996. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science 274, 1527–1531.

Linnet, K., Koed, K., Wiborg, O., Gregersen, N., 1995. Serotonin depletion decreases serotonin transporter mRNA levels in rat brain. Brain Res. 697, 251–253.

Meltzer, H.Y., Lowry, M.T., 1987. The serotonin hypothesis of depression: I. Psychopharmacology. In: H.Y. Meltzer (Ed.), The Third Generation of Progress. Raven Press, New York.

Mirmiran, M., Van de Poll, N.E., Corner, M.A., Van Oyen, H.G., Bour, H.L., 1981. Suppression of active sleep by chronic treatment with clomipramine during early postnatal development: effects upon adult sleep and behavior in the rat. Brain Res. 204, 129–146.

Moret, C., Briley, M., 1996. Effects of acute and repeated administration of citalopram on extracellular levels of serotonin in rat brain. Eur. J. Pharmacol. 295, 189–197.

Murphy, D.L., 1990. Neuropsychiatric disorders and the multiple human brain serotonin receptor subtypes and subsystems. Neuropsychopharmacology 3, 457–471.

Nishikawa, T., Scatton, B., 1985. Inhibitory influence of GABA on central serotonergic transmission. Raphe nuclei as the neuroanatomical site of the GABAergic inhibition of cerebral serotonergic neurons. Brain Res. 331, 81–90.

Pranzatelli, M.R., 1994. Dissociation of the plasticity of 5-HT_{1A} sites and 5-HT transporter sites. Neurochem. Res. 19, 311–315.

Pranzatelli, M.R., Martens, J.M., 1992. Plasticity and ontogeny of the central 5-HT transporter: effects of neonatal 5,7-dihydroxytryptamine lesions in the rat. Dev. Brain Res. 70, 191–195.

Ramamoorthy, S., Cool, D.R., Mahesh, V.B., Leibach, F.H., Melikian, H.E., Blakely, D., Ganapathy, V., 1993. Regulation of the human serotonin transporter. Cholera toxin-induced stimulation of serotonin uptake in human placental choriocarcinoma cells is accompanied by increased serotonin transporter mRNA levels and serotonin transporter-specific ligand binding. J. Biol. Chem. 268, 21626–21631.

Ramamoorthy, J.D., Ramamoorthy, S., Papapetropoulos, A., Catravas, J.D., Leibach, H., Ganapathy, V., 1995. Cyclic AMP-independent up-regulation of the human serotonin transporter by staurosporine in choriocarcinoma cells. J. Biol. Chem. 270, 17189–17195.

Sotelo, C., Cholley, B., El Mestikawy, S., Gozlan, H., Harmon, M., 1990. Direct immunohistochemical evidence of the existence of 5-HT_{1A} autoreceptors on serotoninergic neurons in the midbrain raphe nuclei. Eur. J. Neurosci. 12, 1144–1154.

Spurlock, G., Buckland, P., O'Donovan, M., McGuffin, P., 1994. Lack of effect of antidepressant drugs on the levels of mRNAs encoding serotoninergic receptors, synthetic enzymes and 5-HT transporter. Neuropharmacology 33, 433–440.

Stern, W.C., Johnson, A., Bronzino, J.D., Morgane, P.J., 1981. Neuropharmacology of the afferent projections from the lateral habenula and substantia nigra to the anterior raphe in the rat. Neuropharmacology 20, 979–989.

Vandermaelen, C.P., Aghajanian, G.K., 1983. Electrophysiological and pharmacological characterisation of serotonergic dorsal raphe neurons recorded extracellularly and intracellularly in rat brain slices. Brain Res. 289, 109–119.

Velazquez-Moctezuma, J., Ruiz, O.D., 1992. Neonatal treatment with clomipramine increased immobility in forced swim test: an attribute of animal models of depression. Pharm. Biochem. Behav. 42, 737–739.

Vogel, G., Hartley, P., Neill, D., Hagler, M., Kors, D., 1988. Animal depression model by neonatal clomipramine: reduction of shock induced aggression. Pharmacol. Biochem. Behav. 31, 103–106.

Vogel, G., Neill, D., Hagler, M., Kors, D., 1990a. A new animal model of endogenous depression: a summary of present findings. Neurosci. Biobehav. Rev. 14, 85–91.

Vogel, G., Neill, D., Hagler, M., Kors, D., Hartley, P., 1990b. Decreased intracranial self-stimulation in a new animal model of endogenous depression. Neurosci. Behav. Rev. 14, 65–68.

Welner, S.A., de Montigny, C., Desorches, J., Desjardins, P., Suranyi-Cadotte, B., 1989. Autoradiographic quantification of serotonin (5-HT_{1A}) receptors following long-term anti-depressant treatment. Synapse 4, 347–352.

Wright, D.E., Seroogy, K.B., Lundgren, K.H., Davis, B.M., Jennes, L., 1995. Comparative localisation of serotonin$_{1A,1C,2}$ receptor subtype mRNAs in rat brain. J. Comp. Neurol. 351, 357–373.

Yavari, P., Vogel, G.W., Neill, D.B., 1993. Decreased raphe unit activity in a rat model of endogenous depression. Brain Res. 611, 31–36.