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Persistently Increased Density of Serotonin Transporters in the Frontal Cortex of Rats Treated with Fluoxetine During Early Juvenile Life

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ABSTRACT

This experimental animal study was performed in order to assess possible long-term effects of the administration of the selective serotonin reuptake inhibitor fluoxetine (Prozac®) during early periods of juvenile life on the developing central serotonergic and noradrenergic systems. Fluoxetine was administered via the drinking water (5 mg/kg/day) for a period of two weeks to very young (day 25) and somewhat older (day 50) rats. The effect of this treatment on the density of serotonin and noradrenaline transporters was measured by ligand-binding assays in various brain regions. The B_max-values of [³H]-nisoxetine binding were not affected by either treatment schedule, but a significant increase of the B_max-values of [³H]-paroxetine binding was found in the brains of early fluoxetine-treated rats. This increase was restricted to the frontal cortex and persisted long after the termination of the treatment into adulthood (day 90). The most likely explanation of this observation is a stimulatory effect of the fluoxetine treatment on the outgrowth of serotonergic projections in the frontal cortex of very young rats. This is the first empirical demonstration of long-lasting effects of the administration of a selective serotonin reuptake inhibitor during juvenile life on the maturation of the central serotonergic system.

INTRODUCTION

Because of the possible continuation of child and adolescent psychiatric disturbances into adult psychopathologies, their common psychobiologic background, and many similarities of their behavioral expressions, pharmacological agents with proven efficacy in adult patients are also used for the drug treatment of children and adolescents suffering from such disorders. One example of this trend is the prescription of selective serotonin reuptake inhibitors (SSRIs) in child and adolescent psychiatry (Scahill et al. 1997; Riggs et al. 1997; Kallepalli et al. 1997). Due to their high degree of selectivity and their minimal side effects, SSRIs have widely and successfully been applied in adult psychiatry, e.g., for the treatment of de-

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pression, obsessive-compulsive disorder (OCD), and anxiety or eating disorders (for review see Messiha, 1993). SSRIs inhibit the reuptake of serotonin (5-HT) into presynaptic nerve terminals and show only negligible affinity for 5-HT and other neurotransmitter receptors (Hyttel 1994; Wong et al. 1991). As with other antidepressants, the therapeutic effect of SSRIs is only seen after several weeks of treatment. The neurobiological mechanisms of this delayed clinical response are still poorly understood.

Initially SSRIs attenuate the firing activity of the serotonergic raphe-neurons by overstimulation of their somatodendritic 5-HT1A-receptors. After prolonged exposure to the drug, these autoreceptors become desensitized, and the normal firing activity is restored, resulting in a marked increase of 5-HT release in the terminal fields (Bel and Artigas 1993; Auerbach and Hirvonen 1995). The downregulation of 5-HT-transporter densities and m-RNA-expression observed by several other groups upon long-term administration of SSRIs to experimental animals is thought to additionally contribute to an enhancement of central 5-HT-mediated actions (Lesch et al. 1993; Pineyro et al. 1994).

The increased 5-HT output must be assumed and has already been shown to act as a trigger for secondary adaptive changes, e.g., at the level of expression of individual postsynaptic 5-HT-receptor systems (Wong and Bymaster 1981; Zemlan and Garver 1990), in the responsiveness of other transmitter systems to 5-HT-mediated stimulation (Shachar et al. 1997), or in the stability of the established synaptic connectivity in distant 5-HT projection fields (Mazer et al. 1997). These rather complex long-term changes caused by long-term administration of SSRIs (but also of the less selectively acting tricyclic antidepressants and MAO-inhibitors) are thought to restore a deficiency in or to normalize a disturbed function of the 5-HT-system in, e.g., depression, OCD, anxiety, or sleep disorders.

Indirect evidence for a disturbed 5-HT function is derived from an abnormal hormonal response to 5-HT-agonists (5-HTP, fenfluramine) in children with childhood depression (Ryan et al. 1992; Kaufmann et al. 1998; Birmaher et al. 1997), in aggressive boys (Pine et al. 1997; Halperin et al. 1994), or in boys with ADHD (Halperin et al. 1997; Schulz et al. 1998). The causes of such alterations are not yet completely resolved. Both genetic predispositions and adverse rearing conditions seem to play important roles (Pine et al. 1996; Coplan et al. 1998).

It is assumed that child and adolescent mood disorders are much like and continuous with adult manifestations of these disorders. Especially in child and adolescent depression, noradrenergic and mixed serotonergic/noradrenergic tricyclic antidepressants were found less effective than selective serotonin reuptake inhibitors (Ryan and Varma 1998; Emshie et al. 1997).

Even though the exact mechanism of the long-term therapeutic action of SSRIs (as that of the tricyclic antidepressant drugs) in adult patients is still unresolved, these drugs have been used in children and adolescents for the treatment of many different conditions. Fluoxetine is mostly used for the psychopharmacological treatment of children suffering from depressive syndromes and OCD (De Vane and Sallee 1996; Scahill et al. 1997; Riggs et al. 1997; Kallepalli et al. 1997). In some cases, such treatments were started at rather early ages (younger than 10 years), and doses up to 1 mg/kg per day were administered over periods of several weeks (Geller et al. 1995; Riddle et al. 1992). Side effects were modest, and even the severe symptoms caused by an accidental overdose remitted spontaneously (Riddle et al. 1989). However, single cases of exaggerated extrapyramidal symptoms, and self-injurious or manic behavior during fluoxetine treatment of adolescent patients have been reported (Eisenhauer and Jermain 1993; King et al. 1991; Go et al. 1998). Only a few authors expressed general concerns about differences between young and adult patients in peripheral pharmacokinetics and the maturity of their neuronal networks and transmitter systems involved in cognitive, motor, and emotional regulation (Ryan 1992; Shapiro 1996; Birmaher 1998). Indeed, the multitude of experimental studies on the behavioral and neurobiological effects of psychoactive drugs in adult animal models is contrasted by an amazing paucity of research devoted to uncover the short- and long-term consequences of similar drug treatments during the period of brain maturation in humans.

It is well known from neurodevelopmental studies that during early periods of brain development 5-HT acts as a trophic, developmental signal involved in the regulation of many different aspects of brain maturation (for review see Lauder 1990; Whitaker-Azmitia 1991). Already in the fetal brain, 5-HT is released from outgrowing 5-HT nerve terminals. It plays a critical role in the self-regulation of axonal outgrowth and synaptogenesis of 5-HT projections arising from the 5-HT neurons located in the midbrain raphe nu-
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celi (for review see Azmitia and Whitaker-Azmitia 1991). This developing 5-HT system appears to be particularly sensitive to experimental manipulations of its normal activity and the release of 5-HT by its own terminals. Long-lasting effects on the maturation and later function of the central 5-HT system of experimental animals have been reported, e.g., after increased tryptophan ingestion during pregnancy and later life (Huether et al. 1997), after prenatal fluoxetine exposure (Cabrera-Vera et al. 1997; Cabrera and Battaglia 1994), after neonatal administration of clomipramine (Kinney et al. 1997), or other serotonergic drugs (Frieder and Grimm 1985; Baker and Hoff 1991).

In the course of brain maturation during postnatal life, the initial function of 5-HT as a regulator of the outgrowing 5-HT fibres is gradually replaced by its later predominant role as a neurotransmitter and neuromodulator in central information processing, and the initial plasticity of 5-HT projections is superseded by a more stable innervation pattern of its distal projection fields.

Consequently long-lasting pharmacological manipulations of 5-HT release in the adult brain must be assumed to cause long-lasting adaptations not so much at the level of the established structural architecture of the central 5-HT system but rather at the level of functional regulation of 5-HT output. The reported desensitization of somatodendritic autoreceptors of the raphe neurons (Bel and Artigas 1993; Auerbach and Horth 1995) and downregulation of 5-HT transporters of 5-HT nerve terminals (Pineyro et al. 1994; Lowry 1951) caused by subchonic administration of SSRIs in adult rats are examples of such functional adaptations of the central 5-HT system in the adult brain.

The effects of similar treatments on young animals, however, are difficult to predict. Until now, only two studies have addressed this issue. In a gestational model, Whitaker-Azmitia et al (1994) found that maternal treatment with MAO inhibitor throughout pregnancy caused a decreased density of 5-HT transporters in their offspring. The effect was most pronounced in the cortex and persisted at least until postnatal day 30. In a more recent study, Hansen and Mikkelsen (1998) administered the serotonin reuptake blocker, clomipramine, to suckling rats (day 8–22) and found an impressive increase in the expression of 5-HT transporter m-RNA in the serotonergic raphe neurons by the end of this treatment (day 22). Since the level of 5-HT transporter m-RNA in the serotonergic neurons was shown to be closely related to the formation of 5-HT presynapses (Ivgy-May et al. 1994), this observation suggests that early SSRI treatment may even stimulate 5-HT synaptogenesis in distant projection fields of the midbrain 5-HT-neurons.

Based on these findings, we hypothesized that the long-term administration of SSRIs may affect the maturation of the 5-HT-system, that the expression of a selective marker of 5-HT presynapses should be more affected (the density of 5-HT-transporters as estimated by, e.g., the B$_{max}$-values of $^3$H-paroxetine binding) than that of a marker of noradrenergic presynapses, and that the effects should be more pronounced in younger than in older rats and in later- compared to earlier-maturing projection fields of the central 5-HT-system. In order to clarify this issue, we administered fluoxetine over a period of two weeks to a group of very young (prepubertal) and to a group of somewhat older (pubertal) rats and measured the densities of 5-HT transporters in selected 5-HT projection fields at adulthood.

METHODS

Animal experiments

Animal experiments were performed in accordance with German laws for the care and use of laboratory animals (as approved by the Bezirksregierung Braunschweig, License No. 604.42502/01-24.92).

Wistar rats were obtained from a commercial breeder (Harlan-Winkelmann GmbH, Borchten, Germany) and used for further breeding in our own environmentally conditioned animal facility under standardized conditions. After mating, the dams were housed in single cages with free access to food and water. Litter size was reduced to 8 pups per dam. After weaning the young rats (only males were used in these experiments) were placed in separate cages, two per cage. In one group of rats (N = 12) fluoxetine treatment was started early, at day 25 (the fluoxetine was kindly provided by the Eli Lilly, Indianapolis, IN). The drug was administered via the drinking water for a period of two weeks. Six rats of this early treated group were killed by decapitation, after a drug-free interval of 10 days, at day 50; the other rats of this early treated
group were killed at day 90, i.e., 8½ weeks after discontinuation of the fluoxetine treatment. In a second group of rats (N = 6) the same two-week fluoxetine treatment was started at an older age, at day 50, and the rats were killed at day 90. Littermates served as controls and received normal tap water (N = 6 per group, one group killed at day 50, two groups killed at day 90). The dose of fluoxetine administered daily by the drinking water was adjusted to 5 mg/kg/day based on daily monitoring of amounts consumed by the two rats per cage. These amounts were about 10% of body weight and remained unaffected by the addition of fluoxetine. The steady state plasma concentrations of fluoxetine and its active metabolite, norfluoxetine, achieved by this regimen were measured in separate experiments by high performance liquid chromatography and ultraviolet detection (HPLC-UV) according to Bagli et al. (1997). All rats were killed at noon time, and the brains were quickly removed, dissected, and frozen on dry ice.

Sample preparation

The frozen brain samples (100–150 mg) were homogenized by sonification in a Branson Sonifier (Model: 240, set 5.0, Branson Inc., Danbury, CT) for 10 seconds in 5 volumes of ice-cold PBS (potassium phosphate 10 mM plus 0.9% NaCl, pH 7.4, containing 0.1 mM phenylmethylsulfonyl fluoride (PMSF) and 0.02% thimerosal). The pellets were resuspended in 30 vol. of ice-cold buffer (50 mM Tris/HCl, pH 7.4 containing 120 mM NaCl and 5 mM KCl). After low-speed centrifugation (1000xg, 20 min, 4°C), the supernatants were centrifuged again at 40000xg for 10 min at 4°C. The pellets were washed twice in the same buffer, and the final pellets were resuspended in buffer to give a final concentration of 60–70 mg wet weight/ml. Protein concentrations in ELISA and binding samples were measured by the method of Lowry et al. (1951). Both membrane and supernatant samples were stored at −80°C until further used for [³H]-paroxetine and [³H]-nisoxetine binding assays.

[³H]-paroxetine and [³H]-nisoxetine binding assays

Aliquots of the stored membrane suspensions were further diluted to 15–20 mg wet weight/ml in Tris-buffer (0.2–0.3 mg protein/ml) and incubated for 2 hours at room temperature with [³H]-paroxetine (specific activity: 20.2 Ci/mmole, purchased from New England Nuclear, Boston, MA) in the absence or presence of 200 μM 5-HT (5-hydroxytryptamine hydrochloride, Sigma Chemicals, St. Louis, MO) for the estimation of specific binding. The reaction mixture consisted of 100 μl membrane suspension (20 to 30 μg protein), 100 μl of [³H]-paroxetine-containing buffer (six concentrations covering a range of final ligand concentrations in the assay system from 0.05 nM to 1.00 nM), and 100 μl buffer or 5-HT solution in buffer. For measurements of [³H]-nisoxetine binding, the washed membranes were suspended in Tris-buffer containing 300 mM NaCl and 5 mM KCl and incubated for 60 min at 25°C with [³H]-nisoxetine (specific activity: 20.2 Ci/mmole, purchased from New England Nuclear, Boston, MA) in the absence or presence of 100 μM desipramine (Sigma Chemicals, Saint Louis, MO) for the estimation of specific binding. The reaction mixture consisted of 100 μl membrane suspension (20–30 μg protein), 100 μl of [³H]-nisoxetine-containing buffer (six concentrations covering a range of final ligand concentrations in the assay system from 0.05 to 4.00 μM), and 100 μl buffer or desipramine solution. After incubation, the reaction mixtures were readily filtered through Wathman GF/B filters using a 12-channel cell harvester (Model 11025, Skatron Instruments AS., Lier, Norway), and the filters were washed with 20 vol of ice-cold buffer. The radioactivity trapped by the filters was determined by liquid scintillation spectroscopy.

Data analysis and statistics:

The affinity and capacity parameters (K_D- and B_max-values) of [³H]-paroxetine and [³H]-nisoxetine binding were derived from Scatchard plots of saturation isotherms of specific binding data measured over a concentration range of 0.05 to 1.00 nM by least squares regression analysis (NESS 5.9 software of J. L. Hintze, Kaysville, Utah). Data were expressed as means ± SD. The statistical significance of differences between the means of the binding data measured in the various brain regions of control and experimental animals was tested by ANOVAs followed by two-tailed post-hoc t-test. Results were considered significant for p-values <0.05.
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RESULTS

Both [3H]-paroxetine and [3H]-nisoxetine binding to cortical membrane preparations were very specific, saturable, and of high affinity. The saturation curves were better fitted by a one-site rather than a two-site model, with Hill coefficients very close to 1. Scatchard transformation of the binding data gave a single straight unbroken line, indicating a single apparent class of binding sites with no evidence of cooperativity.

\( K_D \)-values

The estimated affinity parameters (\( K_D \)-values) of the binding sites for both ligands in the various brain regions were rather similar, and no differences were noticed between the \( K_D \)-values measured in these brain regions of pubertal (day 50) and young adult (day 90) rats. Neither the very early nor the rather late fluoxetine administration to juvenile rats affected the \( K_D \)-values of [3H]-paroxetine or [3H]-nisoxetine in any of the brain regions studied (see Table 1).

\( B_{max} \)-values

The measured densities of serotonin and noradrenaline transporters (\( B_{max} \)-values of [3H]-paroxetine and [3H]-nisoxetine binding, respectively) in various brain regions of pubertal and young adult rats and the influence of early and late fluoxetine treatments on these parameters are summarized in Tables 1 and 2. As expected, the densities of 5-HT transporters were higher in the midbrain and the hypothalamus compared to the cortical regions, while this was not the case for noradrenaline transporters. No significant differences in the densities of both transporters in individual brain regions were observed between 50-day and 90-day-old rats. Neither early nor late fluoxetine treatment affected the \( B_{max} \)-values of [3H]-nisoxetine binding in any of the brain regions studied here (Table 2).

The \( B_{max} \)-values of [3H]-paroxetine binding, however, were significantly increased in the frontal cortex of rats following early fluoxetine administration. This increase of 5-HT-transporter density by about 20% was already seen at day 50 and persisted until adulthood.

The \( B_{max} \)-values of [3H]-paroxetine binding in other brain regions of the early fluoxetine-treated rats remained unaffected, and the increased density of 5-HT transporters in the frontal cortex was no longer detected in rats treated with same dose of fluoxetine for the same period at an older developmental age (Table 3).

Plasma levels of drugs, growth, and behavior

The steady-state plasma levels of the fluoxetine and its metabolites, norfluoxetine, achieved by the administration of fluoxetine with the drinking water to either very young or older rats were almost identical (plasma concentration of fluoxetine in young rats: 27.0 ± 13.2 ng/ml, in older rats: 29.9 ± 14.5 ng/ml;

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Age</th>
<th>Controls</th>
<th>Fluoxetine-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>^3H-Paroxetine</td>
<td>day 50</td>
<td>0.050 ± 0.009 μM</td>
<td>0.045 ± 0.012 μM</td>
</tr>
<tr>
<td></td>
<td>day 90</td>
<td>0.047 ± 0.010 μM</td>
<td>0.049 ± 0.008 μM</td>
</tr>
<tr>
<td>^3H-Nisoxetine</td>
<td>day 50</td>
<td>55.37 ± 3.60 μM</td>
<td>54.01 ± 7.13 μM</td>
</tr>
<tr>
<td></td>
<td>day 90</td>
<td>53.66 ± 8.38 μM</td>
<td>53.97 ± 6.38 μM</td>
</tr>
</tbody>
</table>

\( K_D \) = values were measured by ligand binding assays as described in Methods.

Values represent the mean ± SD of 6 rats per group.

No age-related or treatment-induced differences were found also in other brain regions (data not shown here).
WEGENER ET AL.

### Table 2. Effect of Subchronic Fluoxetine Administration on $B_{max}$-Values of $[^3H]$-Nisoxetine Binding in Various Brain Regions of Pubertal Rats (Day 50) and Young Adult Rats (Day 90) After Fluoxetine Treatment

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Frontal</th>
<th>Cortical</th>
<th>Occipital</th>
<th>Subcortical</th>
<th>Midbrain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Parietal</td>
<td></td>
<td>Hypothalamus</td>
<td></td>
</tr>
<tr>
<td>Contr. 1</td>
<td>6</td>
<td>200.9 ± 8.7</td>
<td>213.2 ± 24.6</td>
<td>162.8 ± 41.1</td>
<td>228.4 ± 72.9</td>
<td>226.3 ± 50.7</td>
</tr>
<tr>
<td>Fluox. 1</td>
<td>6</td>
<td>204.8 ± 14.7</td>
<td>172.9 ± 35.5</td>
<td>134.6 ± 50.2</td>
<td>179.8 ± 87.9</td>
<td>196.7 ± 44.7</td>
</tr>
<tr>
<td>Contr. 2</td>
<td>6</td>
<td>223.5 ± 12.9</td>
<td>145.8 ± 27.6</td>
<td>156.6 ± 12.4</td>
<td>165.34 ± 4.8</td>
<td>158.35 ± 26.08</td>
</tr>
<tr>
<td>Fluox. 2</td>
<td>6</td>
<td>264.6 ± 20.8</td>
<td>129.1 ± 5.5</td>
<td>136.7 ± 26.4</td>
<td>237.3 ± 103.8</td>
<td>185.3 ± 47.3</td>
</tr>
<tr>
<td>Contr. 3</td>
<td>6</td>
<td>222.4 ± 25.9</td>
<td>127.9 ± 18.1</td>
<td>154.5 ± 31.3</td>
<td>204.0 ± 69.9</td>
<td>196.0 ± 49</td>
</tr>
<tr>
<td>Fluox. 3</td>
<td>6</td>
<td>202.3 ± 24.5</td>
<td>138.8 ± 32.3</td>
<td>168.7 ± 32.9</td>
<td>194.7 ± 76.7</td>
<td>204.5 ± 51.7</td>
</tr>
</tbody>
</table>

Fluoxetine was administered with the drinking water for a period of two weeks.

Contr. 1 tap water controls, analyzed at day 50
Fluox. 1 fluoxetine treatment started at day 25, analyzed at day 50
Contr. 2 tap water controls, analyzed at day 90
Fluox. 2 fluoxetine treatment started at day 25, analyzed at day 90
Contr. 3 tap water controls, analyzed at day 90
Fluox. 3 fluoxetine treatment started at day 50, analyzed at day 90

$B_{max}$-values were measured by ligand binding assays as described in Methods.

Values represent the mean ± SD of 6 rats per group (fmol $[^3H]$-nisoxetine bound per mg protein).

No significant differences in the density of $[^3H]$-nisoxetine binding sites were found between fluoxetine-treated and control rats.

Plasma concentration of norfluoxetine in young rats: 242.4 ± 58.1 ng/ml; in older rats: 271.8 ± 109.4 ng/ml; all values are means ± SD, N = 4).

The weight gain of the control and fluoxetine-treated groups was monitored each day and was found to increase similarly throughout the study.

No overt behavioral changes were noticed between groups when observed in their home cages.

### Table 3. Effect of Subchronic Fluoxetine Administration on $B_{max}$-Values of $[^3H]$-Paroxetine Binding in Various Brain Regions of Pubertal Rats (Day 50) and Young Adult Rats (Day 90)

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Frontal</th>
<th>Cortical</th>
<th>Occipital</th>
<th>Subcortical</th>
<th>Midbrain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Parietal</td>
<td></td>
<td>Hypothalamus</td>
<td></td>
</tr>
<tr>
<td>Contr. 1</td>
<td>6</td>
<td>808.4 ± 121.8</td>
<td>602.3 ± 133.0</td>
<td>551.1 ± 116.3</td>
<td>1403.6 ± 108.3</td>
<td>1497.6 ± 143.1</td>
</tr>
<tr>
<td>Fluox. 1</td>
<td>6</td>
<td>953.5 ± 148.8*</td>
<td>682.8 ± 231.9</td>
<td>453.2 ± 40.1</td>
<td>1359.2 ± 156.5</td>
<td>1406.2 ± 121.4</td>
</tr>
<tr>
<td>Contr. 2</td>
<td>6</td>
<td>839.1 ± 80.4</td>
<td>553.7 ± 104.5</td>
<td>593.9 ± 174.7</td>
<td>1444.6 ± 236.0</td>
<td>1502.8 ± 201.8</td>
</tr>
<tr>
<td>Fluox. 2</td>
<td>6</td>
<td>983.1 ± 37.5*</td>
<td>671.6 ± 141.7</td>
<td>547.3 ± 139.1</td>
<td>1743.5 ± 124.3</td>
<td>1440.8 ± 84.4</td>
</tr>
<tr>
<td>Contr. 3</td>
<td>6</td>
<td>851.2 ± 45.8</td>
<td>614.3 ± 128.5</td>
<td>564.9 ± 95.20</td>
<td>1527.8 ± 138.8</td>
<td>1599.2 ± 208.8</td>
</tr>
<tr>
<td>Fluox. 3</td>
<td>6</td>
<td>847.8 ± 83.1</td>
<td>618.3 ± 207.6</td>
<td>559.7 ± 111.4</td>
<td>1348.8 ± 141.7</td>
<td>1527.8 ± 212.2</td>
</tr>
</tbody>
</table>

Fluoxetine was administered with the drinking water for a period of two weeks.

Contr. 1 tap water controls, analyzed at day 50
Fluox. 1 fluoxetine treatment started at day 25, analyzed at day 50
Contr. 2 tap water controls, analyzed at day 90
Fluox. 2 fluoxetine treatment started at day 25, analyzed at day 90
Contr. 3 tap water controls, analyzed at day 90
Fluox. 3 fluoxetine treatment started at day 50, analyzed at day 90

$B_{max}$-values were measured by ligand binding assays as described in Methods.

Values represent the mean ± SD of 6 rats per group (fmol $[^3H]$-paroxetine bound per mg protein).

Significant different values of the density of $[^3H]$-paroxetine binding sites were found only in the frontal cortex between Contr. 1 and Fluox. 1 and Contr. 2 and Fluox. 2 (*, $p < 0.05$)
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DISCUSSION

This animal study assessed the possible long-term effects of fluoxetine, given at early periods of brain maturation, on the development of central serotonergic and noradrenergic systems. The results show that the administration of fluoxetine during juvenile life may cause an increase in the density of 5-HT-transporters restricted to a specific distant projection field (i.e., the frontal cortex) of the serotonergic neurons located in the midbrain raphe nuclei. This drug-induced effect may persist until adulthood, i.e., long after the discontinuation of the fluoxetine treatment. In the rat model, the persistently increased 5-HT transporter density was found to be restricted to the frontal cortex. It was only seen in rats which were given fluoxetine by their drinking water very early, i.e., during the first two weeks after weaning, and not in rats subjected to an identical treatment regime during their pubertal period, i.e., at 8 and 9 weeks of age.

The dose of 5 mg/kg is relatively high in comparison to the therapeutic doses used in humans. However, because of the higher hepatic drug-metabolism in rodents, many other drugs are generally administered to rats in about 10-fold higher dosages compared to humans. In adult rats, the minimal dose of fluoxetine required for a significant inhibition of 5-HT uptake was found to be 5 mg/kg (Wong et al. 1983). In subchronic experiments this dose caused an enhancement of stimulated 5-HT overflow in vivo, but no overt behavioral changes or anorectic responses (O'Connor and Krup, 1994). The plasma levels of fluoxetine and norfluoxetine after acute administration of 5 mg/kg were similar to the values measured in the present study (Caccia et al. 1990).

The daily intake of fluoxetine in the early and late treatment groups was nearly identical (about 10% of body weight was consumed daily as fluoxetine-containing drinking water), and so were their plasma levels of fluoxetine and norfluoxetine.

The increased 5-HT transporter density measured in the frontal cortex of early fluoxetine-treated rats may be due to an upregulation of the number of 5-HT transporters expressed in the 5-HT presynapses. Likewise, the early fluoxetine treatment may have stimulated the outgrowth of 5-HT axons and the formation of 5-HT terminals in the frontal cortex. In this case, the increased density of 5-HT transporters would merely reflect the increased 5-HT innervation density in this brain region. For the following reasons, a selective upregulation of the number of 5-HT transporters expressed by the 5-HT nerve terminals in the frontal cortex is not likely: (a) Even though molecular biological studies have provided evidence that the expression of 5-HT transporters may be subject to regulatory phenomena (Ramamoorthy et al. 1993; King et al. 1992), it is difficult to understand how such an upregulation in the intact brain may be restricted to a specific area of the extended distal projection fields of the 5-HT neurons located in the midbrain, and why such upregulation is only seen following early, but not later treatment; (b) the long-term administration of SSRIs to adult rats has consistently been shown to have no effect (Graham et al. 1987; Dewar et al. 1993) or to reduce, but never to increase, the amount of m-RNA of the 5-HT transporter in 5-HT neurons of the raphe nuclei, the number of 5-HT transporters expressed, or the efficacy of 5-HT uptake in the distant projection fields of the raphe neurons (Lesch et al. 1993; Pineyro et al. 1994).

Much more likely, and much better supported by empirical data, is a regionally restricted, selective stimulation of 5-HT axonal growth and synaptogenesis in the frontal cortex by the early fluoxetine treatment. The development of 5-HT neurons, axonal growth, and synapse formation has been shown to be controlled by their own transmitter, 5-HT (for review see Azmitia and Whitaker-Azmitia 1991). The 5-HT released from outgrowing 5-HT nerve terminals is able to activate 5-HT₁A-receptors of neighboring astrocytes and to stimulate thus the production and release of a neurotrophic peptide, S-100β, which acts as growth factor to enhance the elongation, collateral sprouting, and synaptogenesis of 5-HT neurites (Whitaker-Azmitia et al. 1990). The subchronic administration of SSRIs, through the blockade of 5-HT reuptake and the subsequent desensitization of somatodendritic 5-HT₁A-autoreceptors, is known to elevate the steady-state levels of 5-HT in the extracellular space in distant projection fields of the raphe nuclei (Bel and Artigas 1993). It is therefore very likely that the fluoxetine treatment enhanced the 5-HT-induced production and release of the astrocytic growth factor, and therefore, the growth of 5-HT nerve fibers and axon terminals in the frontal cortex of early-treated rats.

It has long been recognized (but largely ignored by many researchers) that the ascending 5-HT afferences are endowed with an enormous degree of structural plasticity even in the adult brain. Collateral sprout-
ing, regeneration, and synaptogenesis have been observed after lesions at different sites along the ascending projections of the 5-HT neurons of the raphe nuclei (Azimtia et al. 1991; Bjorklund et al. 1981; Jacob and Azmitia 1992). Such lesion-induced regeneration and sprouting of 5-HT axons has been shown to result in the formation of new synapses and the functional recovery of 5-HT afferences in the denervated projection fields (Nygren et al. 1974; Wuttke et al. 1977). In addition to the sprouting seen at the proximal stump of damaged axons, collateral sprouting and compensatory changes in 5-HT innervation density have been observed also at other remote sites of the selective 5-HT lesions in the brain (Bjorklund and Stenevi 1979). Especially the nonjunctional, free 5-HT nerve endings in these areas seem to elongate and establish functional connections with target sites (Wiklund and Mollgard 1979). The number of such nonjunctional 5-HT varicosities, and maybe, therefore, the degree of compensatory changes in the 5-HT innervation density, differs between brain regions and is particularly high in the frontal cortex (Seguela et al. 1989). During earlier periods of brain development, the degree of this structural plasticity of the ascending 5-HT projections must be assumed to be higher than at later stages. The observed persisting increase in the density of 5-HT transporters in the frontal cortex of the early fluoxetine-treated juvenile rats is therefore most likely due to an increased density of 5-HT terminals in this brain region. Compatible with this view are observations on the stimulation of 5-HT axonal outgrowth and synaptogenesis in the cortex of adult rats after previous partial lesions of the ascending monoaminergic projections (Huether et al. 1997; Nakamura 1990).

It has long been assumed that the outgrowth of monoaminergic projections in the developing brain is hampered by adverse rearing conditions, lack of attachment, and long-lasting uncontrollable stress during infancy (for review see Huether 1998). Obviously, the developing 5-HT system is much more plastic than originally thought (Goldman-Rakic and Brown 1982). The final density of 5-HT innervation established in the distant projection fields of 5-HT neurons may therefore well be affected, positively and negatively, by social and environmental factors.

The consequences of the presumed 5-HT hyperinnervation resulting from early fluoxetine treatment on cortical information processing are difficult to assess. The dense, predominantly nonjunctional, 5-HT innervation of the cortex is capable of modulating the activity of vast cellular assemblies throughout the cortex (for reviews see Seguela et al. 1989; Spoont 1992). An imbalance in the innervation density of these projection fields may have widespread global influences on brain function. In principle, cortical 5-HT activity acts to constrain neuronal information processing. It stabilizes signal propagation through its inhibitory actions on neuronal activity and prevents impingement of the system by exogenous signal sources. Its activity ensures that only signals of sufficient intensity are able to interfere with current information flow. Under conditions of a reduced 5-HT output, the ability of neuronal networks to maintain the integrity of the signal flow pattern will be impaired and the likelihood of switching to unstable information processing increases. An increased 5-HT-output, as it presumably occurs in the frontal cortex of early fluoxetine-treated rats, may facilitate the formation of regionally focused cycles in information flow. Under such conditions, neuronal information processing is characterized by increased redundant signal propagation and stronger maintenance of subthreshold response patterns (for a more detailed description of the modulatory role of 5-HT in neuronal information processing, see Spoont 1992).

No obvious behavioral alteration was noticed in the early fluoxetine-treated rats. This finding is similar to clinical reports in humans of a lack of significant effects on global behavioral measures following exposure to tricyclic antidepressants or SSRIs in utero (Nulman et al. 1997, Chambers et al. 1996) or during breast-feeding (Wisner et al. 1996). It does not, however, exclude the possibility of subtle changes in impulsivity, open field activity, or learning ability. Such alterations were noticed in adult rats after neonatal exposure to clomipramine, a treatment regime which was proposed to model endogenous depression (Vogel et al. 1990). We have recently shown that in adult rats a similar regionally restricted 5-HT hyperinnervation of the frontal cortex is elicited by olfactory bulbectomy (Grecksch et al. 1997). Such bullectomized rats show subtle behavioral alterations and are widely used as animal models of depression to study the mechanisms of action of antidepressant drugs.

The significance of the observed increased density of 5-HT transporters in the frontal cortex of early fluoxetine-treated rats for SSRI treatment of affective disorders and OCD in child and adolescent psychiatry is difficult to predict. The degree of maturity of the rat brain and the degree of plasticity of its 5-HT projections during the first few weeks after weaning can hardly be related to human brain maturation during
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childhood. One cautious conclusion, however, may be drawn from the present findings: The administration of SSRIs during periods when the central 5-HT system is still rather plastic and capable of structural rearrangements of its distant projections may trigger adaptive responses which are different from those seen in the mature brain and which may persist for long periods after the discontinuation of the treatment, maybe even for the rest of an individual’s lifetime.

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