Effect of Prenatal Fluoxetine (Prozac) Exposure on Brain Serotonin Neurons in Prepubescent and Adult Male Rat Offspring

THERESA M. CABRERA-VERA, FRANCISCA GARCIA, WILFRED PINTO and GEORGE BATTAGLIA

Department of Pharmacology and Experimental Therapeutics, Loyola University of Chicago, Stritch School of Medicine, Maywood, Illinois

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ABSTRACT

The present study examines the consequences of prenatal fluoxetine exposure on brain serotonin [5-hydroxytryptamine (5-HT)] neurons in male offspring. Pregnant rats were administered either saline or fluoxetine (10 mg/kg s.c.) daily from gestational day 13 through gestational day 20. The biochemical status of brain 5-HT neurons was assessed in prepubescent and adult offspring by measuring 1) the 5-HT and 5-hydroxyindoleacetic acid content, 2) the density of [3H]paroxetine-labeled 5-HT uptake sites and 3) the ability of the 5-HT-releasing drug p-chloroamphetamine to reduce 5-HT content. Biochemical parameters were assessed in the frontal cortex, hypothalamus, hippocampus, striatum and midbrain. Comparative effects on dopamine and norepinephrine content in selected regions were also determined. Prenatal exposure to fluoxetine significantly reduced (28%) 5-HT content in the frontal cortex of prepubescent but not adult male offspring. In contrast, in adult progeny prenatal fluoxetine exposure produced a significant decrease only in midbrain 5-HT content (28%). In addition, p-chloroamphetamine markedly reduced 5-HT content in all brain regions examined, but the ability of p-chloroamphetamine to reduce 5-HT content was significantly attenuated only in the midbrain of adult progeny prenatally exposed to fluoxetine. No significant differences were observed between control and fluoxetine-exposed progeny with respect to brain 5-hydroxyindoleacetic acid content, the 5-hydroxyindoleacetic acid/5-HT ratio or the density of 5-HT uptake sites, regardless of the brain region examined or the age of the offspring. These data provide additional evidence that prenatal exposure to fluoxetine can produce limited, rather than global, changes in brain 5-HT neurons and target tissues in fetal progeny. Fluoxetine exposure in the midbrain of adult progeny prenatally exposed to fluoxetine can produce limited, rather than global, changes in brain 5-HT systems remain to be elucidated.

Fluoxetine (Prozac) is a member of the class of antidepressants known as selective serotonin reuptake inhibitors, because it preferentially inhibits the transport of serotonin (5-HT) into presynaptic nerve terminals and exhibits negligible affinity for a number of neurotransmitter receptor subtypes (Peroutka and Snyder, 1980; Thomas et al., 1987; Fuller et al., 1991; Wong et al., 1991). The high degree of selectivity of fluoxetine and its minimal side effects, relative to the tricyclic antidepressants, accounts, in part, for the widespread use of this drug. Consequently, women of childbearing age may constitute a large percentage of the population of patients taking this medication, and the therapeutic use of fluoxetine may continue throughout pregnancy.

Pohland et al. (1989) demonstrated that fluoxetine crosses the placenta and enters fetal brain tissue, where it is likely to act at 5-HT transporters reported to be present and functional in fetal brain (Mercado and Hernandez-R, 1992; Ivgy-May et al., 1994). Because 5-HT plays a critical role in the development of 5-HT neurons and target tissues in fetal brain (Lauder and Krebs, 1978; Chubakov et al., 1986; Whitaker-Azmitia et al., 1987; Azmitia and Whitaker-Azmitia, 1987; Lauder, 1990), exposure of fetal brain to fluoxetine may affect the regulation of fetal brain 5-HT and consequently the normal maturation of brain 5-HT pathways. However, to date few studies have assessed the neurochemical teratogenic potential of this drug. Studies in rats and rabbits indicate that prenatal exposure to fluoxetine, at moderate doses (i.e., doses that are not toxic to the mother), does not produce gross physical abnormalities in the progeny, nor does it affect fetal viability or litter size (Stanford and Patton, 1993; Byrd and Markham, 1994; Cabrera and Battaglia, 1994; Vorhees et al., 1994), suggesting no physical teratogenic effects. Likewise, evaluation using a variety of behavioral paradigms

ABBREVIATIONS: DA, dopamine; GD, gestational day; 5-HIAA, 5-hydroxyindoleacetic acid; HPLC, high-performance liquid chromatography; 5-HT, 5-hydroxytryptamine; NE, norepinephrine; PCA, p-chloroamphetamine; PD, postnatal day.
indicates that prenatal fluoxetine exposure does not produce adverse effects in rat offspring (Hoyt et al., 1989; Vorhees et al., 1994). Few studies have attempted to investigate the neurochemical teratogenic potential of fluoxetine with respect to brain serotonin pathways.

Montero et al. (1990) demonstrated that prenatal exposure to fluoxetine decreased [3H]imipramine binding to presynaptic 5-HT uptake sites in the cortex of prepubescent rat offspring. Subsequently, Romero et al. (1994) reported that prenatal exposure to fluoxetine reduced 5-HT receptor-stimulated phosphoinositide hydrolysis in the cortex of prepubescent, but not adult, progeny. We previously reported that prenatal exposure to fluoxetine reduced the density of 5-HT_2A/2C receptors only in the hypothalamus of adult male offspring (Cabrera and Battaglia, 1994). Consistent with the reduction in hypothalamic 5-HT receptors, the neuroendocrine response to a 5-HT_2A/2C agonist was significantly attenuated in the fluoxetine-exposed progeny, suggesting a functional consequence for the decrease in postsynaptic receptors. Taken together, these studies indicate that prenatal fluoxetine exposure can produce neurochemical and functional alterations in pre- and postsynaptic components of brain 5-HT pathways in rat progeny in the absence of visually apparent physical terata.

The present study investigates the consequences of prenatal fluoxetine exposure on the biochemical status of 5-HT neurons in various brain regions, by measuring 1) the basal serotonin (5-HT) and 5-HIAA content, 2) the density of [3H]pargyline-labeled 5-HT uptake sites and 3) the ability of the presynaptically acting, 5-HT-releasing drug PCA to reduce regional 5-HT content, as previously reported (Fuller et al., 1965; Fuller, 1980, 1992; Kuhn et al., 1985; Adell et al., 1989; Fattaccini et al., 1991). Comparative changes in DA and NE levels were also determined in selected brain regions, as well as the changes in markers of monoamine neurons that occur as a consequence of normal maturation.

Herein we report that prenatal fluoxetine exposure does not produce comparable alterations in 5-HT neurons in all brain regions. The reductions in basal 5-HT levels and the attenuated ability of PCA to reduce 5-HT content in the present study appear to be region-specific and age-dependent. These data are consistent with other findings, from the limited reports available, indicating that prenatal fluoxetine exposure can produce alterations in brain 5-HT neurons in specific brain regions in rat offspring at specific postnatal ages. Although it is presently unknown whether brain 5-HT neurons in human offspring would be affected by prenatal exposure to fluoxetine, comparable vulnerability of 5-HT systems in human offspring may be clinically relevant, because dysfunction of 5-HT pathways has been implicated in the etiology of various psychiatric disorders, including depression, anxiety and aggressive behavior (Siever and Trestman, 1993; Owens and Nemeroff, 1994; Baldwin and Rudge, 1995).

**Methods**

**Animals.** Pregnant Sprague-Dawley rats weighing 280 to 320 g were obtained from Zivic-Miller (Zelienople, PA) and maintained in a facility with controlled temperature (22–24°C), humidity (50–55%) and illumination (12/12-hr light/dark cycle, lights on at 7 A.M.). The determination of GD 0 was carried out by the supplier and was defined by the presence of a copulatory plug. All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, as adopted and promulgated by the National Institutes of Health.

Pregnant rats arrived in the laboratory on GD 5. Although we previously reported that our treatment paradigm does not alter maternal weight gain during pregnancy (Cabrera and Battaglia, 1994), all experimental animals were placed on a nutritionally balanced liquid diet (Cabrera et al., 1993), starting on GD 8 and continuing throughout the injection period, to monitor daily nutritional intake. Experimental dams received a single daily injection of either 0.9% saline (2 ml/kg s.c.) or fluoxetine (10 mg/kg/2 ml s.c.), beginning on GD 13 and ending on GD 20. After termination of the injection paradigm, all animals had free access to food and water.

All offspring from each of the experimental groups were fostered to untreated lactating dams, to eliminate the possible influence of drug-induced differences in nurturing. All pups were weaned on PD 21.

The number of samples (n) for each postnatal treatment group was obtained by using a single pup from each of the litters. Progeny were sacrificed by decapitation, as described below, at PD 26 or PD 70. These ages represent pre- and postpubescent time points, respectively.

**Assessment of the functional status of serotonergic nerve terminals.** Offspring were sacrificed 1 hr after receiving a single injection of either saline or the 5-HT releaser PCA (5 mg/kg i.p.). The brains were quickly removed and placed on a cold petri dish, and the hypothalamus, striatum, hippocampus, midbrain and frontal cortex were dissected out. The brain regions were immediately placed in cryovials, frozen in liquid nitrogen and stored at −70°C until used for HPLC analysis of monoamine content or radioligand binding analysis of 5-HT uptake sites. Basal levels of 5-HT and its primary metabolite 5-HIAA were determined in each brain region from animals receiving the saline injection before sacrifice. As an index of 5-HT turnover, the ratio of 5-HIAA to 5-HT values was determined for all acutely saline-challenged animals from both prenatal treatment groups (Karsaet et al., 1994). For comparative purposes, DA and NE contents were determined simultaneously with 5-HT and 5-HIAA levels.

**HPLC determination of biogenic amines.** HPLC determination of brain biogenic amines was carried out as described by Saller and Salama (1984), with some modifications. Brain regions were sonicated in 10 volumes of ice-cold 0.1 N perchloric acid containing 0.5 μM dihydroxybenzylamine as the internal standard used to calculate the recovery of the biogenic amines. The homogenate was then centrifuged at 20,000 × g for 15 min at 4°C. Twenty-five to fifty microliter aliquots of the supernatant were injected into an HPLC system. The HPLC system consisted of a delivery pump (model 501; Waters, Marlborough, MA) in conjunction with a Waters 717 autosampler and an analytical column (Microsorb C18, 5 μm, 150 mm × 4.6 mm;Rainin, Woburn, MA) protected by a guard column (Microsorb C18, 5 μm, 15 mm × 4.6 mm;Rainin). An electrochemical detector (model LC-4C; Bioanalytical Systems, West Lafayette, IN) with a glassy carbon electrode was used at a voltage setting of +0.75 V vs. an Ag/AgCl reference electrode. The mobile phase was composed of 9 g/liter monochloracetic acid, 0.25 mM EDTA, 0.375 g/liter 1-octanesulfonic acid and 1% tetrahydrofuran (pH 3.0). The solvent flow was maintained at 2.0 ml/min. Standard solutions of 5-HT, 5-HIAA, NE and DA were prepared in ice-cold 0.1 N perchloric acid containing 0.5 μM dihydroxybenzylamine. The system was run by Millennium 2010 Chromatography Manager, a computer program that performs data acquisition, processing and management of chromatographic information (Waters). Tissue precipitates were resuspended in 0.1 N NaOH to achieve a tissue concentration of approximately 30 mg/ml, and then 20-μl aliquots were taken for protein determination according to the method of Lowry et al. (1951). For each brain region, samples from prepubescent and adult progeny were assayed simultaneously for brain monoamine content.

**Radioligand binding assay for 5-HT uptake sites.** Regional 5-HT uptake sites were measured in the cortex, hippocampus, stri-
atum and midbrain according to a previously published protocol (Battaglia et al., 1987), using a single saturating concentration of radioligand. This method is sensitive to changes in the maximal density of uptake sites. The density of hypothalamic 5-HT uptake sites in fluoxetine-exposed offspring was previously reported (Cabrera and Battaglia, 1994). Determination of the maximal density of 5-HT uptake sites was carried out in a 5.0-ml assay containing 1 mg wet weight of tissue and a single saturating (20 × Kᵢ) (Battaglia et al., 1987) concentration (0.4 nM) of [³H]paroxetine (20 Ci/mmol) in 50 mM Tris-HCl (pH 7.7, 25°C), 120 mM NaCl, 5 mM KCl. Non-specific binding was determined in the presence of 1.0 μM citalopram. Tubes containing drugs and tissue were incubated for 120 min at room temperature and then filtered rapidly over Whatman GF/C filters that had been presoaked in 0.5% polyethyleneimine. The samples were then washed with 20 ml of 50 mM Tris-HCl (pH 7.7, 25°C). Filters were then added to scintillation vials containing 5 ml of Ultima Gold (Packard Instrument Co., Downers Grove, IL) scintillation fluid. The vials were shaken for 60 min, and samples were counted for 2.5 min on a Beckman LS5000TD scintillation counter at an efficiency of 60%. For each brain region, samples from prepubescent and adult progeny were assayed simultaneously.

Materials. NE hydrochloride, DA hydrochloride, serotonin creatinine sulfate and 5-HIAA free salt were obtained from Research Biochemicals International (Natick, MA). Monochloroacetic acid, 1-octanesulfonylic acid sodium salt and tetrahydrofuran were obtained from J.T. Baker (Phillipsburg, NJ). [³H]Paroxetine was obtained from New England Nuclear (Boston, MA). Citalopram was provided by Lundbeck (Copenhagen, Denmark). Fluoxetine was generously provided by the Eli Lilly Co. (Indianapolis, IN), PCA and all other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

Statistics. The data are represented as the group means and the S.E.M. Statistical analysis of the data was performed by a two-way analysis of variance. Individual group means were compared by Newman-Keuls test (Steel and Torrie, 1960), using a computer program (SigmaStat; Jandel, San Rafael, CA). P < .05 was chosen as the level of significance.

Results

Site-Specific Reductions in Monoamine Content in Fluoxetine-Exposed Offspring

5-HT and 5-HIAA. Table 1 reports basal 5-HT levels in several brain regions in prepubescent and adult male progeny prenatally exposed to either saline or fluoxetine. Prenatal exposure to fluoxetine significantly reduced 5-HT content (−28%) in frontal cortex only in prepubescent male progeny. 5-HT content was not altered in either the hypothalamus, hippocampus, striatum or midbrain in fluoxetine-exposed offspring at PD 26. In contrast, in adult animals, basal 5-HT content was significantly reduced only in the midbrain (−28%) of fluoxetine-exposed offspring (table 1). Basal 5-HT content in frontal cortex, hypothalamus, hippocampus and striatum was comparable in control and fluoxetine-exposed adult offspring. In contrast to the selective reductions in 5-HT content in fluoxetine-exposed prepubescent and adult progeny, basal 5-HIAA content was not altered by prenatal exposure to fluoxetine. As shown in table 2, 5-HIAA content in frontal cortex, hypothalamus, hippocampus, striatum and midbrain was similar in control and fluoxetine-exposed progeny, at both prepubescent and adult ages.

In addition to the determination of regional basal 5-HT and 5-HIAA content, the 5-HIAA/5-HT ratio was calculated as an index of 5-HT turnover (Karstaedt et al., 1994). As shown in table 3, prenatal fluoxetine exposure did not produce any significant changes in this index of serotonin turnover in any of the brain regions examined (frontal cortex, hypothalamus, hippocampus, striatum and midbrain), regardless of the age of the animal.

Catecholamines. Basal DA and NE levels were determined for comparative purposes and are shown in tables 4 and 5, respectively. Prenatal fluoxetine exposure did not alter basal DA levels in the hypothalamus, striatum or midbrain in either prepubescent or adult male offspring (table 4). The effects of prenatal fluoxetine exposure on DA levels in the frontal cortex and hippocampus could not be determined because the basal levels were below the detectable limit of our assay. As shown in table 5, basal NE content was not altered by prenatal fluoxetine exposure in either prepubescent or adult male offspring in any of the brain regions examined (frontal cortex, hypothalamus, hippocampus, striatum and midbrain).

Effect of Prenatal Fluoxetine Exposure on the Ability of a 5-HT-Releasing Drug to Reduce Regional 5-HT Content

A single injection of PCA resulted in significant (P < .05) decreases in 5-HT content in all brain regions examined at both postnatal times (fig. 1). The magnitude of the reduction in 5-HT differed as a consequence of brain region and postnatal age (20–67% reductions), with the greatest reduction in 5-HT content being observed in the frontal cortex of prepubescent male offspring (fig. 1). At PD 26, PCA administra-

### Table 1

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Frontal cortex</th>
<th>Hypothalamus</th>
<th>Hippocampus</th>
<th>Striatum</th>
<th>Midbrain</th>
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<tr>
<td>Saline</td>
<td>23.4 ± 1.1 (7)</td>
<td>45.5 ± 1.8 (8)</td>
<td>14.9 ± 0.8 (7)</td>
<td>20.1 ± 2.4 (8)</td>
<td>40.6 ± 1.8 (7)</td>
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<td>45.3 ± 2.8 (7)</td>
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<td>19.2 ± 1.6 (8)</td>
<td>45.5 ± 4.7 (8)</td>
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</tr>
<tr>
<td>Saline</td>
<td>27.3 ± 1.8 (7)</td>
<td>49.5 ± 3.0 (8)</td>
<td>18.0 ± 0.8 (8)</td>
<td>22.2 ± 1.2 (8)</td>
<td>65.6 ± 8.2 (8)†</td>
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<tr>
<td>Fluoxetine</td>
<td>31.1 ± 2.3 (8)†</td>
<td>48.2 ± 3.0 (8)</td>
<td>18.3 ± 1.7 (8)</td>
<td>20.8 ± 1.7 (8)</td>
<td>47.1 ± 2.3 (8)*</td>
</tr>
</tbody>
</table>

* Significantly different from control values within the respective brain region (P < .05).
† Significantly different from the respective values obtained in prepubescent progeny (P < .05).


**TABLE 2**

Basal 5-HIAA levels in prepubescent and adult male progeny prenatally exposed to fluoxetine

Data represent the means ± S.E.M. from seven or eight saline-challenged rats per group, with each rat within a group being obtained from a different litter. The number of rats per group is shown in parentheses. Prenatal exposure to fluoxetine (10 mg/kg/2 ml s.c., to dams from GD 13 to GD 20) did not alter basal 5-HIAA content in any of the brain regions examined. Data from each brain region were analyzed separately, using two-way analysis of variance, followed by Newman-Keuls post hoc test.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Frontal cortex</th>
<th>Hypothalamus</th>
<th>Hippocampus</th>
<th>Striatum</th>
<th>Midbrain</th>
</tr>
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<tbody>
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<td></td>
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</tr>
<tr>
<td>Saline</td>
<td>0.06 ± 0.09 (8)*</td>
<td>0.57 ± 0.15 (8)*</td>
<td>0.81 ± 0.11 (7)*</td>
<td>1.06 ± 0.04 (8)*</td>
<td>0.69 ± 0.07 (8)*</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>0.69 ± 0.14 (8)*</td>
<td>0.57 ± 0.08 (8)*</td>
<td>0.81 ± 0.11 (7)*</td>
<td>1.06 ± 0.04 (8)*</td>
<td>0.69 ± 0.07 (8)*</td>
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<tr>
<td>Adult</td>
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</tr>
<tr>
<td>Saline</td>
<td>0.67 ± 0.09 (8)*</td>
<td>0.56 ± 0.15 (8)*</td>
<td>0.78 ± 0.06 (7)*</td>
<td>1.01 ± 0.05 (8)*</td>
<td>0.59 ± 0.15 (8)*</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>0.69 ± 0.14 (8)*</td>
<td>0.57 ± 0.08 (8)*</td>
<td>0.81 ± 0.11 (7)*</td>
<td>1.06 ± 0.04 (8)*</td>
<td>0.69 ± 0.07 (8)*</td>
</tr>
</tbody>
</table>

* Significantly different from the respective values obtained in prepubescent progeny (P < .05).

**TABLE 3**

Basal serotonin turnover in prepubescent and adult male progeny prenatally exposed to fluoxetine

Data represent the means ± S.E.M. of 5-HIAA/5-HT ratios from seven or eight saline-challenged rats per group, with each rat within a group being obtained from a different litter. The number of rats per group is shown in parentheses. Prenatal exposure to fluoxetine (10 mg/kg/2 ml s.c., to dams from GD 13 to GD 20) did not alter basal 5-HT turnover in the frontal cortex, hypothalamus, hippocampus, striatum or midbrain of prepubescent male progeny. Likewise, 5-HT turnover was not altered in adult male progeny across any of the brain regions examined. Data from each brain region were analyzed separately, using two-way analysis of variance.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Frontal cortex</th>
<th>Hypothalamus</th>
<th>Hippocampus</th>
<th>Striatum</th>
<th>Midbrain</th>
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<tr>
<td>Saline</td>
<td>0.99 ± 0.11 (8)</td>
<td>0.79 ± 0.05 (8)</td>
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<td>Fluoxetine</td>
<td>1.28 ± 0.06 (8)</td>
<td>0.76 ± 0.10 (7)</td>
<td>1.54 ± 0.04 (8)</td>
<td>1.67 ± 0.07 (8)</td>
<td>1.23 ± 0.04 (8)</td>
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<tr>
<td>Adult</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Saline</td>
<td>0.67 ± 0.09 (8)*</td>
<td>0.56 ± 0.15 (8)*</td>
<td>0.78 ± 0.06 (7)*</td>
<td>1.01 ± 0.05 (8)*</td>
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<tr>
<td>Fluoxetine</td>
<td>0.69 ± 0.14 (8)*</td>
<td>0.57 ± 0.08 (8)*</td>
<td>0.81 ± 0.11 (7)*</td>
<td>1.06 ± 0.04 (8)*</td>
<td>0.69 ± 0.07 (8)*</td>
</tr>
</tbody>
</table>

* Significantly different from the respective values obtained in prepubescent progeny (P < .05).

**TABLE 4**

Basal DA levels in prepubescent and adult male progeny prenatally exposed to fluoxetine

Data represent the means ± S.E.M. from six to eight saline-challenged rats per group, with each rat within a group being obtained from a different litter. The number of rats per group is shown in parentheses. DA levels were beyond the detection limits of the assay in the cortex and hippocampus. Prenatal exposure to fluoxetine (10 mg/kg/2 ml s.c., to dams from GD 13 to GD 20) did not alter basal DA content in the hypothalamus, striatum or midbrain of prepubescent male progeny. Likewise, DA content was not altered in adult male progeny across any of the brain regions examined. Data from each brain region were analyzed separately, using two-way analysis of variance, followed by Newman-Keuls post hoc test.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Hypothalamus</th>
<th>Striatum</th>
<th>Midbrain</th>
</tr>
</thead>
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<tr>
<td>Prepubescent</td>
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</tr>
<tr>
<td>Saline</td>
<td>26.2 ± 1.7 (8)</td>
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<td>8.7 ± 0.3 (7)</td>
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<td>Fluoxetine</td>
<td>25.1 ± 1.5 (6)</td>
<td>400.1 ± 25.0 (8)</td>
<td>7.8 ± 0.4 (7)</td>
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<tr>
<td>Adult</td>
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</tr>
<tr>
<td>Saline</td>
<td>28.1 ± 1.1 (7)</td>
<td>741.4 ± 25.1 (7)*</td>
<td>7.8 ± 0.8 (7)</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>27.0 ± 1.9 (8)</td>
<td>718.7 ± 42.4 (8)*</td>
<td>8.1 ± 0.4 (7)</td>
</tr>
</tbody>
</table>

* Significantly different from the respective values obtained in prepubescent progeny (P < .05).

Prenatal exposure to fluoxetine did not alter the density of 5-HT uptake sites in prepubescent male progeny. In contrast, in adult offspring, PCA significantly reduced midbrain 5-HT content in both progeny groups, but the magnitude of the reduction was significantly less in progeny of fluoxetine-exposed dams (fig. 1B). In contrast to the attenuated responses observed in midbrain, in other brain regions (i.e., frontal cortex, hippocampus, striatum and hypothalamus) significant but comparable PCA-induced reductions in 5-HT content were obtained in control and prenatal fluoxetine-exposed adult offspring.

**Effect of Prenatal Fluoxetine Exposure on the Density of 5-HT Uptake Sites**

Prenatal exposure to fluoxetine did not alter the density of 5-HT uptake sites in prepubescent male progeny in either the frontal cortex, hippocampus, striatum or midbrain (table 6). Likewise, in adult progeny, no alterations in 5-HT uptake site density were observed as a consequence of prenatal fluoxetine exposure in any of the brain regions examined (table 6).

**Age-Related Changes in the Functional Status of 5-HT Neurons in Control Progeny**

**Basal monoamine content.** Several differences in basal monoamine content were observed as a consequence of normal maturation. For example, midbrain 5-HT levels were significantly greater in adult control progeny than in prepu-
basement control progeny (+61%) (table 1). In contrast, 5-HIAA content was significantly lower in adult control progeny, compared with values for preschool control animals, in the hypothalamus, hippocampus, striatum and midbrain but not in the frontal cortex (table 2). These differential changes in 5-HT and 5-HIAA levels as a consequence of maturation resulted in significantly greater 5-HIAA/5-HT ratios (an index of 5-HT turnover) in adult control animals vs. their preschool counterparts, in all brain regions examined (table 3). With respect to age-dependent changes in catecholamines, basal DA levels were greater in striatum, but not in hypothalamus or midbrain, in adult control progeny (table 4). In contrast, NE levels in striatum were comparable at both postnatal ages. However, NE levels were significantly elevated in frontal cortex, hypothalamus, hippocampus and midbrain in adult offspring (table 5).

**PCA-induced reduction of 5-HT content.** In saline-exposed progeny, the ability of PCA to reduce 5-HT content was significantly greater in the frontal cortex of preschool offspring, in comparison with their adult counterparts (fig. 1). In contrast, PCA reduced 5-HT content in midbrain to a greater extent in adult than in preschool animals (fig. 1). However, reductions in 5-HT content in the hypothalamus, hippocampus and striatum after PCA administration were comparable between preschool and adult animals.

**Discussion**

The present study demonstrates reductions in brain 5-HT content only in frontal cortex and midbrain in progeny after prenatal exposure to fluoxetine. The reductions in 5-HT, in the absence of changes in the number of 5-HT transporters in various brain regions, indicate that this effect was not likely due to gross changes in 5-HT innervation (Descarries et al., 1995). However, the attenuated response to a 5-HT releaser in midbrain of adult progeny indicates possible changes in 5-HT transporter function in this brain region produced by prenatal exposure to fluoxetine. Taken together, these data indicate that prenatal exposure to fluoxetine did not produce widespread or global changes in 5-HT neurons. Rather, the effects of prenatal exposure to fluoxetine on brain 5-HT systems were limited to selected brain regions at specific developmental ages. Because only gross brain regions were investigated in the present study, it is possible that prenatal exposure to fluoxetine could have produced discrete changes in 5-HT parameters in specific neuroanatomic loci that could not be detected using homogenate binding assays. Consistent with this possibility, autoradiographic data from our laboratory (Cabrera et al., 1995) have revealed a significant increase in 5-HT₂A/₂C receptor density specifically in the entorhinal cortex of adult fluoxetine-exposed offspring. However, changes in 5-HT₂A/₂C receptors were not detectable when measured in cortical homogenates of adult progeny prenatally exposed to fluoxetine (Cabrera and Battaglia, 1994).

Although the decreases in 5-HT content in adult frontal cortex represent changes in 5-HT axons and terminals, it is unclear from the present data whether the reduction of 5-HT in midbrain represents altered 5-HT in axons/terminals or perikarya. The reduction of 5-HT in frontal cortex and midbrain could reflect a decrease in the synthesis of 5-HT, because these changes occurred in the absence of any alterations in 5-HIAA. It is interesting that the magnitude of the reduction (−28%) in 5-HT was comparable in both brain regions (i.e., frontal cortex and midbrain). However, it is not clear from the present data why prenatal fluoxetine exposure produces reductions in 5-HT only in frontal cortex and midbrain, which occur at different postnatal developmental ages. In contrast to the changes in 5-HT, prenatal exposure to fluoxetine did not alter basal DA or NE levels in any of the brain regions examined, suggesting that catecholamine neurons may be less sensitive to perturbation by prenatal exposure to fluoxetine.

In prenatal fluoxetine-exposed progeny, another notable difference between the frontal cortex and midbrain concerns the ability of the 5-HT releaser PCA to reduce 5-HT content. In frontal cortex, PCA-induced reductions in 5-HT were comparable in control and prenatal fluoxetine-exposed progeny, regardless of developmental age. In contrast, in midbrain of prenatal fluoxetine-exposed progeny, the ability of PCA to reduce 5-HT was attenuated only in adult offspring, at which developmental time the basal 5-HT was 28% lower than values in control progeny. However, it is unlikely that the attenuated response in midbrain is due specifically to the reduced 5-HT levels in midbrain, because 5-HT was also significantly reduced by 28% in frontal cortex, where there was no attenuation in the response to PCA. One possibility is that 5-HT transporter function may be differentially affected in frontal cortex vs. midbrain. PCA enters 5-HT neurons via the 5-HT transporter and facilitates the release of 5-HT.
uptake sites (either collectively or as the number of sites/neurons) could be due to 1) changes in the density of 5-HT exposed adult progeny. Impaired entrance of PCA into 5-HT and/or release processes in 5-HT perikarya in fluoxetine-midbrain, are consistent with an impairment in uptake and attenuated response to the 5-HT releaser PCA only in midbrain perikarya. Differences in the response to PCA could be atattenuated response to PCA only in midbrain perikarya. Differences in the response to PCA could be at 2) changes in the activity (i.e., $K_m$) or maximal transport velocity (i.e., $V_{max}$) of PCA for the 5-HT transporter. Likewise, because PCA-mediated 5-HT release is mediated primarily via a reversal of the 5-HT transport mechanism (Rudnick and Wall, 1992), changes in 5-HT transporter kinetics ($V_{max}$ and $K_m$) and/or density could affect the ability of PCA to reduce 5-HT. However, there was no overall change in the density of 5-HT uptake sites in homogenates of midbrain, or any other brain region, in fluoxetine-exposed progeny. Therefore, the attenuated response to PCA is unlikely to be the result of decreases in 5-HT transporter density, unless such changes were restricted to 5-HT transporters discretely localized on perikarya in dorsal and median raphe. Initial in vitro autoradiographic data from our laboratory indicate no changes in 5-HT transporter density in dorsal and median raphe regions after prenatal exposure to fluoxetine (unpublished observations). The absence of changes in the density of 5-HT uptake sites, as reported herein, does not preclude the possibility that prenatal exposure to fluoxetine may have affected the activity of 5-HT transporters in midbrain. Miller and Hoffmann (1994) have shown that treatments that alter the activity of the 5-HT transporter can do so independently of changes in the density of 5-HT uptake sites.

Another possible explanation for the attenuated PCA response in midbrain may be that prenatal exposure to fluoxetine may have altered the size of the releasable pool of 5-HT. The majority of evidence suggests that PCA releases 5-HT primarily from the cytoplasmic pool of 5-HT found within 5-HT terminals (Sanders-Bush and Martin, 1982; Kuhn et al., 1985; Adell et al., 1989; Rudnick and Wall, 1992). A reduction in the releasable pool of 5-HT may occur independently of changes in the amount of 5-HT stored in secretory vesicles. This would account for the differences noted in PCA effects between frontal cortex and midbrain, despite the comparable reductions in 5-HT content in the two brain regions. Because 5-HT content and the response to PCA were both attenuated in midbrain of adult progeny, it is possible that prenatal exposure to fluoxetine could have altered 5-HT transporter function as well as 5-HT synthesis and/or storage.

In contrast to the midbrain, PCA reduced 5-HT in frontal cortex to a comparable extent in control and fluoxetine-exposed progeny, at both prepubescent and adult ages. The most parsimonious explanation for these findings is that, in the frontal cortex, uptake and release processes and/or the cytoplasmic (releasable) pool of 5-HT may not be affected by prenatal exposure to fluoxetine, regardless of whether basal 5-HT content is reduced. The reduction of basal 5-HT content in frontal cortex may be due to a reduction in the amount of 5-HT stored in secretory vesicles. Similarly, in other brain regions devoid of changes in basal 5-HT (i.e., hypothalamus, hippocampus and striatum), the ability of PCA to reduce 5-HT content was not affected by prenatal exposure to fluoxetine. This suggests that neither the amount of cytoplasmic 5-HT nor the uptake or release processes in these brain regions were altered by prenatal fluoxetine exposure.

Relatively few studies have quantitatively examined age-dependent changes in monoamine content. With respect to catecholamines, the data from the present study are consistent with the reports by Giorgi et al. (1987) and Schwabe et al. (1992), who observed significantly greater striatal DA levels in adult offspring, in comparison with prepubescent

Fig. 1. Reduction in brain 5-HT content (expressed as a percentage of control values) after a single injection of either saline or the 5-HT releaser PCA (5 mg/kg i.p.) in prepubescent (A) and adult (B) male progeny prenatally exposed to either saline or fluoxetine (10 mg/kg administered s.c. to dams from GD 13 to GD 20). Base-line levels of 5-HT for each brain region and treatment group are shown in table 1. Data represent group means ± S.E.M. for six to eight rats per group, with each rat within a group being obtained from a different litter. PCA significantly (P < .05) reduced 5-HT content in all brain regions and across all treatment groups. PCA produced similar reductions in 5-HT content in both control and fluoxetine-exposed prepubescent offspring in the frontal cortex, hippocampus, striatum and midbrain. In contrast, in adult offspring the magnitude of the reduction in 5-HT content after PCA administration was significantly less in midbrain than in fluoxetine-exposed offspring, in comparison with their respective control group. Data were analyzed by two-way analysis of variance, followed by a Newman-Keuls test. CTX, frontal cortex; HYPO, hypothalamus; HIPP, hippocampus; STR, striatum; MDBR, midbrain. †, Significantly different from the reduction in midbrain 5-HT content observed in adult control offspring (P < .05). ††, Significantly different from the respective values in prepubescent control offspring (P < .05).
animals. Similarly, the elevations in basal NE content in adult vs. prepubescent animals, across several brain regions, are consistent with a previous report (Nomura et al., 1976). However, unlike the age-dependent changes in striatal DA content, hypothalamic and midbrain DA levels were similar in prepubescent and adult progeny. With respect to serotonergic markers, age-dependent differences in basal 5-HIAA levels were observed in the hypothalamus, hippocampus and midbrain, with values being significantly lower in adults than in prepubescent progeny (table 2). These data are consistent with a report by Schwabe et al. (1992), who observed lower 5-HIAA, but not 5-HT, levels in the striatum of adult offspring, in comparison with prepubescent progeny. The present study also demonstrates a selective increase in midbrain 5-HT content with maturation, which is consistent with a previous report in whole brain (Nomura et al., 1976). Despite these site-specific and age-dependent changes in 5-HT and 5-HIAA, no significant age-dependent differences were observed in 5-HT uptake sites in any of the gross brain regions examined. However, in control progeny, age-dependent differences were observed in the magnitude of the PCA-induced reductions in 5-HT in frontal cortex and midbrain. To our knowledge, the present study represents the first published report of age-dependent differences in the ability of the serotonin releaser PCA to reduce 5-HT content in various regions of rat brain and the consequences of prenatal fluoxetine exposure on this response.

In summary, the present study demonstrates reductions in rat progeny 5-HT content, limited to frontal cortex and midbrain, after prenatal exposure to fluoxetine. These data suggest age-dependent and site-specific, rather than global, changes in serotonin neurons produced by prenatal fluoxetine exposure. Whereas cortical 5-HT content is reduced in prepubescent fluoxetine-exposed progeny and returns to control values in adult offspring, the reduction in midbrain 5-HT content becomes apparent only after maturation of the offspring. Likewise, the ability of PCA to reduce midbrain 5-HT content is altered only in adult offspring of fluoxetine-exposed dams. These data suggest that the consequences of prenatal fluoxetine exposure on 5-HT neurons may be influenced by maturational processes, as previously reported for 5-HT2A/2C receptors and receptor-mediated responses (Cabrera and Battaglia, 1994). Although it is unknown whether prenatal exposure to fluoxetine would affect the development of 5-HT pathways in human offspring, region-specific and age-dependent reductions in brain 5-HT content and 5-HT neuronal function in human offspring may be of clinical significance, because dysfunction of 5-HT pathways has been implicated in the etiology of various clinical disorders (Siever and Trestman, 1993; Owens and Nemeroff, 1994; Baldwin and Rudge, 1995).

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Send reprint requests to: George Battaglia, Ph.D., Department of Pharma-

cology, Loyola University of Chicago, Stritch School of Medicine, 2160 South First Ave., Maywood, IL 60153.