# Noradrenergic and Serotonergic Neuroendocrine Responses in Prepubertal, Peripubertal, and Postpubertal Rats Pretreated With Desipramine and Sertraline

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**ABSTRACT**

**Objective:** To explore whether developmental status of neurotransmitter systems may affect response to antidepressant treatment. This study investigated whether younger animals, compared with mature animals, showed the same neuroendocrine response to challenge drug probes when pretreated with a serotonergic or noradrenergic antidepressant. **Method:** Prepubertal, pubertal, and adult rats were pretreated with low- or high-dose sertraline or desipramine for 14 days. Animals were then challenged with a noradrenergic probe (clonidine for desipramine-treated animals) or a serotonergic probe (fenfluramine for sertraline-treated animals). The neurohormonal response of growth hormone to the clonidine challenge and prolactin to the fenfluramine challenge was then measured. **Results:** In animals challenged with fenfluramine, the postpubertal control group showed a significantly higher prolactin response to fenfluramine than postpubertal animals pretreated with low- or high-dose sertraline. No differences were found in the pubertal or prepubertal group. In animals challenged with clonidine, there was a significant age by treatment interaction effect for the prepubertal group pretreated with high doses of desipramine (less growth hormone secretion) but not for the peri- or postpubertal groups. **Conclusions:** These data indicate neurodevelopmental factors may play a role in the functional physiology of neurotransmitter systems, which in turn may affect response to psychotropics. *J. Am. Acad. Child Adolesc. Psychiatry*, 2002, 41(8):999–1006. **Key Words:** antidepressant, desipramine, development, growth hormone, prolactin, sertraline.

Child and adolescent major depressive disorder (*DSM-IV*) is a significant mental health and social problem, with prevalence rates estimated up to 8% (Birmaher et al., 1996). The disorder can have vast ramifications on the outcomes for social, scholastic, and occupational aspects of the depressed person's life. It is therefore important to find effective treatments. For unknown reasons, children and adolescents respond poorly to antidepressant therapies, which are effective in adult patients.

Many studies of tricyclic antidepressants (TCAs) have been unable to distinguish active drug from placebo condition in children (Geller et al., 1999) even tough TCAs are effective treatments for depression in adult populations (Kutcher et al., 1994). Selective serotonin reuptake inhibitors (SSRIs) have shown greater efficacy than TCAs; Emslie et al. (1997) determined that the SSRI fluoxetine was superior to placebo in the treatment of pediatric depression, and Keller et al. (2001) found that paroxetine was significantly superior to both imipramine and placebo on certain but not all primary outcome measures in a study of adolescent depression.

Even in these well-designed studies, variations in response rates may be affected by methodological issues such as inadequate dosage and high placebo responders (Birmaher et al., 1996). Another source of variation may be neurodevelopmental pharmacokinetic and pharmacodynamic factors. From the latter perspective, Geller

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et al. (1996) suggest that the age difference in the mechanism of drug response by children and adults following TCA administration is due to the relative maturity of the noradrenergic neurotransmitter system. Thus the efficacy of antidepressant therapy may reflect developmental differences in the neurobiology of the brain.

One approach to testing the integrity or sensitivity of specific developing neurotransmitter systems in animals is through challenge drugs that stimulate specific neurotransmitter systems which in turn elicit characteristic hormonal responses (neuroendocrine challenge tests). The available data on drug provocation tests in children with psychiatric disorders indicate that their neurobiology may be different from that of adults. The challenge drug, fenfluramine, is an amphetamine derivative capable of stimulating the release of serotonin while blocking its reuptake. By increasing serotonin in the synapse, the short-term administration of fenfluramine causes a dose-dependent release of the pituitary hormone prolactin (Power and Cowen, 1992; Van de Kar, 1991). Sallee et al. (2000) studied the serotonergic response of anxious children to a fenfluramine challenge. The obsessive-compulsive disorder (OCD) subset of their sample had a greater prolactin response to fenfluramine compared with controls or children with other anxiety disorders. These findings were in contrast to adults with OCD, who usually show a blunted prolactin response to the fenfluramine challenge (Lucey et al., 1992), indicating a possible overactivity of the serotonergic system in younger subjects. Halperin et al. (1997) studied the prolactin response to fenfluramine challenge in aggressive youths. They found that the younger sample of aggressive boys (younger than 9.1 years) had greater prolactin responses than age-matched controls, a finding not detectable in the older sample. Because aggressive adults showed a blunted prolactin response to fenfluramine challenge (O'Keane et al., 1992), Sallee et al. (2000) postulated that in children with these conditions, the serotonergic system may be overactive at an early stage but supplanted later on with decreased central serotonin function and postsynaptic receptor hypersensitivity in the adult.

In conditions in which noradrenergic overactivity is implicated such as in panic disorder or generalized anxiety disorder, challenge with clonidine elicited a blunted growth hormone response in adults (Abelson et al., 1991). Usually a short-term challenge with clonidine, an $\alpha_2$-presynaptic adrenergic agonist, elicits a growth hormone pulse. Blunted growth hormone response occurs as a result of long-term locus ceruleus activity due to autoreceptor subsensitivity and down-regulation of hypothalamic $\alpha_2$-adrenergic receptors which mediate growth hormone release. In contrast, children with anxiety disorders did not show blunting of growth hormone response to clonidine challenge, especially if they had OCD symptoms (Sallee et al., 1998). The authors explain this finding by suggesting that adrenergic postsynaptic down-regulation may not be a marker of childhood anxiety.

Developmental models that use animals of different ages exposed to long-term pretreatment with antidepressants and then challenged with noradrenergic or serotonergic drug probes could yield valuable information on the underlying status of developing neurotransmitter systems. Long-term treatment (14 days) with desipramine desensitized noradrenergic receptors in adult rats, leading to a blunted growth hormone response to short-term clonidine challenge (O'Donnell and Grealy, 1992), probably reflecting postsynaptic down-regulation. In this study, we hypothesized that immature animals would not show desensitization of adrenergic receptors, i.e., growth hormone response would not be blunted to short-term clonidine challenge in younger animals pretreated with desipramine. We hypothesized that long-term sertraline administration in immature animals would not desensitize the serotonergic receptors to short-term challenge with fenfluramine, i.e., prolactin response to fenfluramine would not be blunted in the immature animals pretreated with sertraline. Desipramine and sertraline were selected because each drug selectively blocks uptake in either the noradrenergic system (Tang and Seeman, 1980) or serotonergic system (Koe et al., 1983), the two major neurotransmitter systems postulated to be involved in the etiology of pediatric depression. Even though desipramine is rarely used in clinical practice, other norepinephrine reuptake blockers such as reboxetine are already used for the treatment of depression in clinical practice for adults. For all drug pretreatment conditions, a low- and high-dose condition was used because reuptake blockade varies with the dosage used (Pineyro and Blier, 1999).

## METHOD

### Animals

One hundred sixty-eight Sprague-Dawley male rats were obtained from Charles River (St. Constant, Quebec) at 19 days ($n$ = 56), 39 days ($n$ = 56), or 80–100 days of age ($n$ = 56). Animals were housed in pairs in three climate-controlled colony rooms under a 12:12 light-dark cycle with lights on from 7:00 A.M. to 7:00 P.M. Pellet food

(Rodent Laboratory Chow, Ralston Purina International) and water were available ad libitum throughout the experiment. Animals which were 19 days old upon arrival were classified as "prepubertal," 39 days old as "pubertal," and 80–100 days old as "adult." Experimental techniques were authorized by the University Committee on Laboratory Animals (protocol 97-059).

### Experimental Design

After 1-day habituation to the animal facility, animals within each age group were randomly assigned to one of five drug treatment conditions—saline (0.9% NaCl, $n = 24$); desipramine (3 mg/kg, $n = 8$); desipramine (15 mg/kg, $n = 8$); sertraline (2 mg kg/kg, $n = 8$); or sertraline (10 mg/kg, $n = 8$)—and received daily intraperitoneal injections for 14 days. A period of 2 weeks was chosen because this is the time required for down-regulation of postsynaptic receptors or desensitization of autoreceptors (Pineyro and Blier, 1999). The animals were removed from their cages and anesthetized by intraperitoneal injection of 65 mg/kg sodium pentobarbital (Somnotol) and then underwent a cardiac cannulation procedure to allow for subsequent drug challenges to be given intravenously and for serial blood sampling in conscious and unrestrained animals. The surgical procedures used were similar to those described by others (Grealy and O'Donnell, 1991; Harms and Ojeda, 1974). After recovery from surgery (3–4 days), animals were transferred to the neuroendocrine laboratory 24 hours in advance of challenge testing to allow habituation of stress hormone levels after disturbance. Blood samples (0.4 mL) were taken from the animals into syringes and immediately placed into chilled Microtainer 1.0-mL tubes with K2-EDTA (Becton Dickinson, Rutherford, NJ). Prechallenge blood samples were taken at 9:30 A.M. (–30 minutes) and 9:55 A.M. (–5 minutes). The challenge drug (clonidine [50 $\mu g\ kg^{-1}$], fenfluramine [7.5 $mg\ kg^{-1}$]) or 0.9% NaCl was administered intravenously at 10:00 A.M. via the cannula in volumes of 0.5 mL. Drug challenge occurred at 10:00 A.M. to control for circadian fluctuations in growth hormone secretion, inasmuch as plasma growth hormone concentrations are cyclic in nature, with first peak occurring just after "lights on" at 7:00 A.M. and first trough beginning 3 hours later. After the challenge, blood samples were withdrawn at 10:05 A.M. (5 minutes), 10:20 A.M. (20 minutes), 10:35 A.M. (35 minutes), 10:50 A.M. (50 minutes), and 11:05 A.M. (65 minutes). All blood samples were immediately centrifuged (12 minutes at 10,000 × *g*) for plasma separation. Plasma was isolated and transferred into 2-mL Micro Tubes (Sarstedt, Germany) and frozen at –70C. Information regarding our hormone assay protocols is available on the *Journal*'s Web site at *www.jaacap.com* via Article Plus.

### Statistical Analyses

For each experimental condition, analyses proceeded in three steps. First, change scores were computed by subtracting the –30-minute time point from the 5-minute postchallenge time point such that positive values on these change scores indicate higher levels for time point 5, negative values indicate higher levels for time point –30, and zero indicates no difference between –30 and 5. Second, change scores were analyzed with univariate analyses of variance (ANOVAs), with age and treatment as factors. Results were divided for each hormone change score by contrasting fenfluramine or clonidine challenge with either saline treatment or drug (sertraline or desipramine) treatment inasmuch as animals in the challenge condition (no treatment but hormone challenge) needed to be compared with those in the saline condition and animals in the treatment condition (low- or high-dose sertraline or desipramine) needed to be compared with animals in the challenge condition with no treatment (summarized in Table 1). Third, significant effects were followed up using simple-effects tests and pairwise comparisons (with Bonferroni corrections). All these comparisons were within each age group and then repeated between age groups in order to draw out meaningful developmental differences. Change scores were graphed as a function of age and treatment for both prolactin (Fig. 1) and growth hormone (Fig. 2)

## RESULTS

### Prolactin

*Saline.* The effect of fenfluramine challenge compared with saline was examined with a 2 (age: 20 days versus 80 days) × 2 (treatment: saline-saline versus saline-fenfluramine) ANOVA with change in prolactin level between –30 and 5 as the dependent measure (owing to technical problems, data were not available for the saline challenge in the 40-day-old group). Results showed a significant main effect of treatment ($F_{1,20} = 12.97$, $p < .01$) and a significant age × treatment interaction ($F_{1,20} = 5.98$, $p < .05$). The significant interaction was followed up in two ways. First, simple-effects tests were used to examine the effect of treatment within each developmental level. Results showed a significant effect of treatment for 80-day-old animals ($F_{1,20} = 18.28$, $p < .001$), but not for 20-day-old animals ($F < 1.0$). As shown in Figure 1, the change in prolactin for 80-day-old animals was significantly higher for saline-fenfluramine than for saline-saline, but there was no significant difference between conditions for 20-day-old

**TABLE 1**
Change in Prolactin Hormone to Fenfluramine Provocation and Change in Growth Hormone to Clonidine Provocation in Animals Pretreated With Saline or Low/High Dose Sertraline or Desipramine

| | Prolactin | | | Growth Hormone | | |
|---|---|---|---|---|---|---|
| | | | | | Saline-Clonidine vs. | |
| Age | Saline-Saline vs. Saline-Fenfluramine | Saline-Fenfluramine vs. High/Low Dose Sertraline | High vs. Low Dose Sertraline | Saline-Saline vs. Saline-Clonidine | High/Low Dose Desipramine | High vs. Low Dose Desipramine |
| 20 days | $p$ = NS | $p$ = NS | $p$ = NS | $p$ = NS | $p < .001$ | $p < .01$ |
| 40 days | NA | $p$ = NS | $p$ = NS | $p$ = NS | $p$ = NS | $p$ = NS |
| 80 days | $p < .001$ | $p < .05$ | $p$ = NS | $p$ = NS | $p$ = NS | $p$ = NS |

*Note:* NS = not significant; NA = not available.

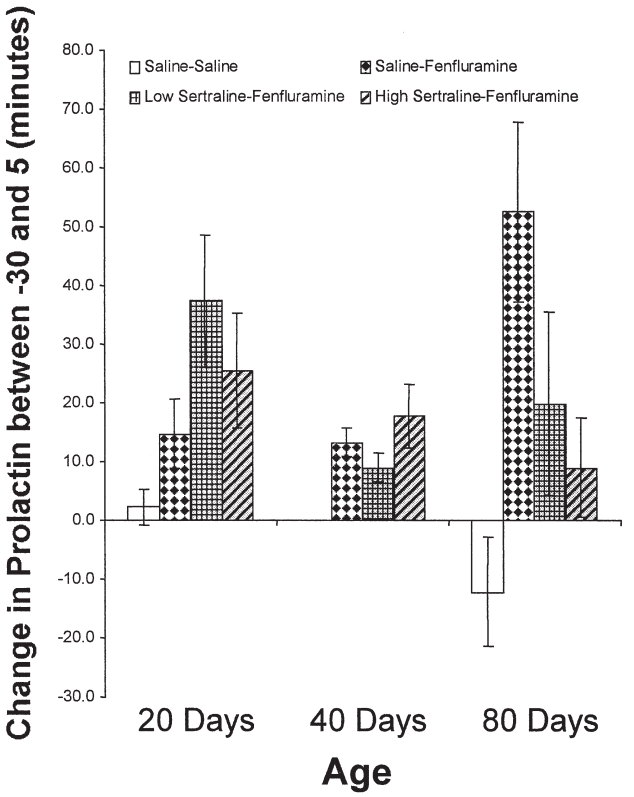


**Fig. 1** Change in prolactin scores as a function of age and treatment. Error bars represent ± 1 SEM. Because of equipment problems, data were not available for 40-day-old rats in this condition.

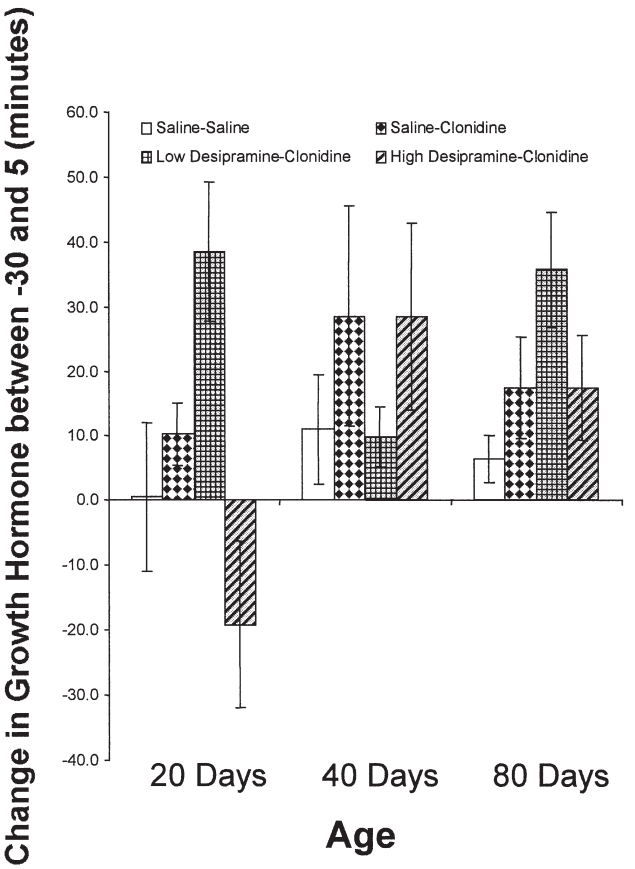


**Fig. 2** Change in growth hormone scores as a function of age and treatment. Error bars represent ± 1 SEM.

animals. Second, simple-effects tests were used to examine the effect of treatment between developmental levels. Results showed that the 20- and 80-day-old animals differed in the saline/fenfluramine condition ($F_{1,20} = 7.53$, $p < .05$), but not in the saline/saline condition ($F < 1$).

*Sertraline.* The effect of fenfluramine following a low and high dose of sertraline was examined with a 3 (age: 20 days versus 40 days versus 80 days) × 3 (treatment: saline-fenfluramine versus low sertraline-fenfluramine versus high sertraline-fenfluramine) ANOVA with prolactin level as the dependent measure. There was a marginal treatment × age interaction ($F_{4,55} = 2.04$, $p = .10$). This interaction was followed up in two ways. First, simple-effects tests were used to examine the effect of treatment within each developmental level. Results showed a significant effect of treatment for the 80-day-old animals ($F_{2,20} = 3.41$, $p < .05$), but not for other animals ($F$ values < 1.0). As depicted in Figure 1, pairwise comparisons of treatments for the 80-day-old animals showed that the saline/fenfluramine condition differed significantly from the low sertraline ($p < .05$) and high sertraline ($p < .05$) conditions, but that the low and high sertraline conditions did not differ from each other. Second, simple-effects tests were used to examine the effect of treatment between developmental levels. There was a significant effect of age for saline-fenfluramine ($F_{2,55} = 3.49$, $p < .05$), but not for other treatments ($F$ values < 1.4). As shown in Figure 1, pairwise comparisons of treatments showed that the 80-day-old saline/fenfluramine animals differed from their 40- and 20-day-old counterparts ($p < .05$).

### Growth Hormone

*Saline.* The effect of clonidine compared with saline was examined with a 3 (age: 20 days versus 40 days versus 80 days) × 2 (treatment: saline-saline versus saline-clonidine) ANOVA with growth hormone as the dependent measure. There were no significant effects ($F < 1.7$; $p > .20$).

*Desipramine.* The effect of clonidine following a low and high dose of desipramine was examined with a 3 (age: 20 days versus 40 days versus 80 days) × 3 (treatment: saline-clonidine versus low desipramine-clonidine versus

high desipramine-clonidine) ANOVA with growth hormone as the dependent measure. Results showed a significant age × treatment interaction ($F_{4,73} = 3.06$, $p < .05$). The significant interaction was followed up two ways. First, simple-effects tests were used to examine the effect of treatment within each developmental level. Results showed a significant effect of treatment for animals 20 days old ($F_{2,73} = 6.05$, $p < .001$), but not for animals 40 or 80 days old ($F < 1.1$). As shown in Figure 2, pairwise comparisons of treatments for 20-day-old animals showed significant differences between low desipramine-clonidine and high desipramine-clonidine animals ($p < .01$), with the saline animals falling between the two groups and marginally different from each ($p < .10$). Second, simple-effects tests were used to examine the effect of treatment between developmental age levels. Results showed a significant effect of age for the high desipramine-clonidine condition ($F_{2,73} = 4.27$, $p < .05$), but not for the saline-clonidine or low desipramine-clonidine conditions ($F$ values < 2.2). As shown in Figure 2, pairwise comparisons showed that the 20-day-old group differed significantly from the 40-day-old group but not from the 80-day-old group. The 40-day-old and 80-day-old groups also did not differ.

## DISCUSSION

The present results clearly indicate developmental differences in the functional physiology of the serotonergic and noradrenergic systems as shown by neuroendocrine responses to challenge drug probes. Concerning the serotonergic system, the adult group showed a significantly more vigorous prolactin response to fenfluramine challenge and suppression of this response when pretreated with low- or high-dose sertraline, compared with the pubertal or the prepubertal group. This confirmed our original hypothesis that mature animals would be able to down-regulate or desensitize postsynaptic serotonergic receptors in response to a serotonin reuptake inhibitor such as sertraline. A decreased sensitivity of postsynaptic 5-HT-1A receptors (Simonovic et al., 1984) or 5-HT-2A/2C receptors (Coccaro et al., 1996) is a possible explanation for this down-regulation inasmuch as these receptors are thought to mediate prolactin release. An alternative explanation is that 5-HT-1A somatodendritic autoreceptors may have been desensitized because of the sustained administration of sertraline. These autoreceptors are activated in response to an increase in extracellular 5-HT such as that produced after the short-term administration of an SSRI, but the inhibition can be overcome by long-term (14–21 days) treatment with low-dose SSRIs (Bel and Artigas, 1993; Pineyro and Blier, 1999).

However, other types of autoreceptors such as the terminal 5-HT-1B/1D autoreceptors may be involved. These receptors have been shown to inhibit 5-HT release and can also be desensitized by long-term paroxetine treatment, but evidence exists that they may require a more complete blockade of 5-HT reuptake sites before desensitization occurs (El Mansari and Blier, 1996; Pineyro and Blier, 1996). This may in part explain why we did not see a difference between adult animals pretreated with low- or high-dose sertraline, as the long-term treatment may not have been long enough to desensitize both populations of receptors. Ultimately it may be the interplay between these different types of autoreceptors that determines the ceiling 5-HT neurons establish on levels of extracellular 5-HT, because after prolonged 5-HT blockade an increase in extracellular 5-HT occurs in the face of a reduction in total 5-HT tissue content (Caccia et al., 1992; Trouvin et al., 1993).

The 20- and 40-day-old animals had lower secretion of prolactin to fenfluramine challenge compared with the adult group, and this did not change as a result of pretreatment with low- or high-dose sertraline pretreatment. A developmental explanation for the younger animals' lack of response to either stimulation or reuptake blockade may be that 5-HT receptors are not fully functional, as the 5-HT stimulatory effect on prolactin as mediated through the 5-HT-1A receptor is not fully developed until at least day 25 (Bero and Kuhn, 1987). Autoregulation of 5-HT neurons involving feedback loops between somatodendritic and terminal autoreceptors mediating intracellular and extracellular events as well as heteroregulation involving more distant feedback loops reflecting noradrenergic, dopaminergic, glutamatergic, and GABAergic inputs to 5-HT neurons may still be in evolution. This is consistent with serotonin's dual role in development as a neurotransmitter and trophic factor (Whitaker-Azmitia, 1991). In addition, extraneuronal influences such as the secretion of nerve growth factors by astroglial cells in immature organisms (Whitaker-Azmitia and Azmitia, 1994) could affect 5-HT responsiveness to challenge or reuptake blockade.

Other developmental neuropharmacological evidence for the delayed ontogeny of the 5-HT system comes from

McCracken and Poland (1995), who found that prepubertal animals did not respond as vigorously as adult animals in their prolactin response when both groups were pretreated with amitriptyline for 10 days and then challenged with the serotonin agonist 1-(*m*-trifluoromethylphenyl) piperazine. They speculated that immature animals might show a reduced capacity to develop functional enhancement of certain 5-HT systems after repeated antidepressant administration. Teicher and Baldessarini (1987) found that the administration of imipramine, a TCA with serotonergic reuptake blockade properties, produced sedation only in rats older than 4 weeks of age. They hypothesized that the absence of imipramine-induced sedation in young rats was due to the delayed development of the serotonin-mediated inhibitory response.

For the noradrenergic system, pretreatment with low-dose desipramine produced a greater growth hormone pulse than pretreatment with high-dose desipramine in the prepubertal group. Low-dose desipramine may not desensitize adrenergic receptors, more specifically presynaptic $\alpha_2$-receptors due presumably to the immaturity of the noradrenergic system in the younger animals. In the adult, presynaptic $\alpha_2$-receptors control the release of noradrenaline through autoinhibition. Hypothalamic $\alpha_2$-receptors control the release of growth hormone–releasing hormone and subsequently release of growth hormone. The noradrenergic system of the younger animal could fail to desensitize either through lack of inhibition or overall increased tone of the noradrenergic impulses. An age-related decline in noradrenergic tone is consistent with the role of the locus ceruleus in plasticity and adaptability in such important functions as control of sleep-wake cycle and gating of vigilance (Aston-Jones et al., 2000). An age-related decline in the activity of noradrenergic neurons in the locus ceruleus has been documented (Olpe and Steinmann, 1982). The development of inhibition through somatodendritic or dendrodendritic $\alpha_2$-autoreceptors has been shown to be present from birth on noradrenergic neurons but is not fully functional until postnatal day 9 and increases with age thereafter (Kimura and Nakamura, 1987). In contrast to low-dose desipramine, high-dose desipramine produces suppression of growth hormone secretion in prepubertal animals. In this age group there may be a need for greater reuptake blockade before there is postsynaptic desensitization. Delayed ontogeny of adrenergic autoreceptors is consistent with the clinical observation of the effects of clonidine causing increased locomotor activity in rats up to 14 days of age, no effect at day 20, and dose-dependent sedation afterward (Reinstein and Isaacson, 1977).

### Clinical Implications

Whereas these animal data cannot be compared directly to the human situation and more specifically to human models of psychopathology, the findings indicate important developmental differences in the ontogeny of the serotonergic and noradrenergic systems that in turn could modulate sensitivity to psychopharmacological therapy. There is important evidence for the developmental modulation of serotonin function with age. Chugani et al. (1997), examining serotonin synthesis rates, found that children from ages 3 months to 3 years had a 200% increase from adult values and this persisted until age 5, when it began to drop to adult levels. Saxena (1995) and Biegon and Gruener (1992) reported a developmental progression of serotonin receptor densities. Responsivity of the serotonergic system to antidepressant treatment could be affected by relative maturity (prepubescent, peripubescent, or postpubescent). Current medication trials usually involve wide variations in age, which may overlap several developmental periods. Simultaneous measures of sex steroid hormones or other indices of physical development are rarely part of medication trial protocols.

Variations in neurotransmitter development not only fluctuate with maturity, but can be gender- and brain region-specific. Andersen et al. (1997) found a significant increase in the number of D1 and D2 striatal receptors and D1 receptors in the nucleus accumbens of periadolescent male rats. The receptor levels were pruned back when the rats reached adulthood, and the changes occurred only in males. Knowledge of these receptor changes could help explain why drugs interacting with these receptors such as stimulants or neuroleptics would either not be effective in this age group or alternatively could lead to a different side effect profile than that of younger animals or have gender effects. With neuroleptics, adolescent males are at increased risk for dystonic reactions compared with adults or children (Richardson et al., 1991).

It is only recently that investigators have begun to study the effect of commonly prescribed pediatric psychotropics in animal models of neuroreceptor development. Moll et al. (2001) reported that methylphenidate administration to young rats (day 25) at a dosage comparable with that used in humans (2 mg/kg) for 14 days caused a persistent reduction in the density of striatal dopamine trans-

porters by 25% at day 45 (adolescence) and 50% at day 70 (adulthood). Wegerer et al. (1999) found that 14 days' administration of fluoxetine at 5 mg/kg to 25-day-old rats caused a significant increase in paroxetine receptor binding in the frontal cortex which persisted into adulthood (day 90). These experiments indicate that psychotropics administered during one period of the lifespan may have enduring effects that persist in adulthood.

### Limitations

Although animal models allow greater control of experimental conditions, they are limited in their generalizability to the human situation. There are species differences in receptor type and distribution, and humans are raised in far more complex environments than laboratory animals, an important factor in brain development. We also did not use animal models of psychopathology with specific deficiencies in neurotransmitter systems, which may mimic more closely psychopathological states in humans. The utility of pharmacological probes is limited even in animal models because of multiple systems involved and lack of pharmacological specificity for the probes themselves (Sallee et al., 1998). Nonetheless, with investigators reporting a dramatic increase in the use of psychotropics in preschool children (Zito et al., 2000), who are in a vulnerable period of brain development, the use of animal models to test neurotransmitter development could lead to valuable insights into novel treatments or approaches.

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