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Developmental Regulation of the Dopamine D_1 Receptor in Human Caudate and Putamen

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Perturbations in the developmental regulation of the dopaminergic system have been hypothesized to participate in the age-dependent onset of schizophrenia. Although data from studies of non-human primates suggest that dopamine D_1-like receptors decrease during adolescence, less information is available concerning changes in human brain. The present study employed quantitative receptor autoradiography to measure D_1-like receptor density and affinity in human caudate and putamen. Samples were obtained postmortem from 15 subjects (9 weeks to 49 years), and grouped a priori *into three classes: infants, adolescents, and adults. Receptor density and affinity were assessed by saturation binding with [^{3}H]-SCH23390, a D_1 receptor antagonist. A decrease in D_1 receptor density was observed from infancy to adulthood, with no change in receptor affinity. The temporal pattern of D_1-like receptor expression during maturation may play a role in the interaction of dopamine with other neurotransmitter systems, and in the occurrence and pharmacotherapy of neurological and neuropsychiatric disorders.* ***[Neuropsychopharmacology 21:641–649, 1999]*** *© 1999 American College of Neuropsychopharmacology. Published by Elsevier Science Inc.*

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There is a large body of data suggesting that some subtypes of schizophrenia may be disorders of neural development. The preclinical course of the disease provides one source of evidence for this contention. Many patients with schizophrenia show subtle signs of the illness long before the onset of grossly psychotic episodes. These patients, who typically are diagnosed during late adolescence, often display a history of motor, social, and cognitive abnormalities during early childhood (Fenton and McGlashan 1991; Harrison 1997a; Lieberman et al. 1997).

A number of structural abnormalities found in the brains of people with schizophrenia provide additional support for a role of developmental processes in this disorder (Walker and Neumann 1994; Weinberger 1995; Wolf and Weinberger 1996; Harrison 1997a). Postmortem studies of patients with schizophrenia have shown altered glial and neuronal densities in different regions of the cortex and nucleus accumbens (Pakkenberg 1990; Benes et al. 1991; Harrison 1997b). Moreover, neuroimaging studies have reported decreased cortical volumes in the frontal and temporal lobes, and enlarged lateral and third ventricles of patients diagnosed with schizophrenia (Chua and McKenna 1995; Kotrola and Weinberger 1995; Pfefferbaum and Marsh 1995). Since the clinical symptoms of schizophrenia ordinarily do not become apparent until adolescence, normal developmental processes occurring during this time may be one of the important neurobiologic bases affecting the emergence of symptoms (Feinberg 1982; Weinberger 1987; Keshavan et al. 1994).

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The dopamine hypothesis of schizophrenia has been one of the major foci of inquiry into the biological underpinnings of the disease (Meltzer and Stahl 1976; Seeman 1987; Davis et al. 1991). This hypothesis originally was derived from two major lines of pharmacological evidence. The first was the impressive correlation between the clinical potency of the early antipsychotics with their affinity for what we now call the D_2 dopamine receptors (Creese et al. 1976). The second line of evidence was provided by the observation that large doses of amphetamines, a drug class known to act at presynaptic dopaminergic terminals, cause psychosis in patients with no previous history of psychotic episodes (Angrist and Gershon 1970). Yet, the actual role and location of dopaminergic dysfunction in the etiology of schizophrenia is less clear. The prefrontal cortex is thought to be involved (Grace 1991), and prevailing evidence suggests that the basal ganglia may also contribute through alterations in the circuitry that encompasses both brain regions (Graybiel 1997).

Studies of dopamine receptor development in rodents have shown that dopamine D_1 receptors undergo age-specific decreases in density (Gelbard et al. 1989; Henry et al. 1986; Ricci et al. 1995; Teicher et al. 1995). Consistent with these findings are data from human and non-human primates showing a similar decrease between perinatal and adult periods (Rinne 1987; Seeman et al. 1987; Palacios et al. 1988; De Keyser et al. 1990; Rinne et al. 1990; Lidow and Rakic 1992; Boyson and Adams 1997). A major limitation of these studies in humans, however, is the small sample size in those time periods that are hypothesized to be critical to the onset of schizophrenia. (i.e., late childhood and early adolescence). In the largest single study of dopamine receptor development in humans, Seeman et al. (1987) provided suggestive evidence for a complex relation between D_1 receptor density and age. This study reported a dramatic increase in dopamine D_1 receptor density from infancy to middle and late childhood and an apparent normalization by young adulthood. D_1 receptor density thereafter exhibited a slow decline throughout the remainder of the lifespan. Seeman et al. (1987) did not evaluate any samples from the central period of adolescence (12–19 yrs) although this would be presumed to be a time period marked by a precipitous drop in D_1 receptor density to achieve normalization of receptor levels by young adulthood.

The goal of the present study was to investigate the normal development of dopamine D_1 receptor density and affinity in the human caudate and putamen. Our study was prompted by the limitations of prior research in this area, especially with regards to the under-representation of adolescence. Receptor affinity is an issue that is examined rarely in developmental studies, but one that may be of great importance. Previous developmental studies in rats found no differences in receptor affinity with age (Broaddus and Bennett 1990; Ricci et al. 1995), however, this has not been studied extensively in humans and primates. Our study, therefore, was designed to investigate not only possible changes in receptor density, but also changes in D_1 receptor affinity during human development. We employed quantitative receptor autoradiography of the D_1 receptor antagonist [^{3}H]-SCH23390 in postmortem samples of caudate and putamen from subjects of varying age, including infants, adolescents and adults. An understanding of the developmental expression of dopamine D_1 receptors in normal brain will provide a basis for evaluating the role of this receptor subtype in schizophrenia and other neurodevelopmental disorders where dopaminergic dysfunction is implicated.

METHODS

Materials

[^{3}H]-SCH23390 was synthesized as described previously (Wyrick and Mailman 1985). R(+)-SCH23390, mianserin, and *cis*-flupenthixol were obtained from Research Biochemicals International (Natick, MA).

Tissue Preparation

Blocks of left human caudate putamen were obtained at autopsy under the authority of the State Medical Examiner of North Carolina in Chapel Hill, NC. Two additional samples (right caudate and putamen of subjects O and P) were obtained from the Brain and Tissue Bank for Neurodevelopmental Disorders at the University of Maryland, Baltimore. The blocks were frozen on dry ice and stored at -80°C. Sections were sliced coronally (16 μm) and thaw-mounted onto gelatin-coated slides. Slides were desiccated at room temperature for 20–24 hours and stored at −80°C until the day of the assay. The age of subjects in this study (n = 15) ranged from nine weeks to 49 years old (for subject history refer to Table 1). These subjects were divided *a priori* into three groups: infants (n = 4, mean age = 10.5 months); adolescents (n = 6, mean age = 16.8 years); and adults (n = 5, mean age = 39 years).

Quantitative Receptor Autoradiography

The radiolabeled antagonist [^{3}H]-SCH23390 was used to detect D_1 receptor binding. For each subject, a series of eighteen adjacent 16 μm sections was assayed. Two brain sections were used to define total binding and one to define nonspecific binding, at each of the six radioligand concentrations. On the day of the assay, slide-mounted tissue sections were defrosted for 15 minutes at room temperature and pre-incubated in assay buffer (50 mM Hepes, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$,

Table 1. History of human subjects used in the study

Subject	Age (yrs, unless specified)	Gender	Ethnicity	Cause of death	Postmortem interval (hrs)
A	47	M	C	MI	11
B	30	M	AA	Hypoxia	25
C	49	M	AA	MI	24
E	39	F	C	MI	13
F	18	F	AA	Congenital Heart Disease	15
G	22 mo	F	AA	Flu/dehydrat.	9
H	9 wk	F	C	SIDS	24
I	15 mo	F	C	SIDS	13
J	16	M	C	Cardiomyopathy	18
K	3 mo	M	C	SIDS	10
L	30	F	C	Sudden Cardiac Arrest	24
M	20	M	C	GSW	12
N	17	M	AA	GSW	18
O	15	M	C	Drowned	20
P	15	M	AA	Auto Accident	13

The deaths of all subjects were deemed accidental. The symbols denote: (gender) F = female, M = male; (ethnicity category) AA = African American, C = Caucasian; (cause of death) MI = myocardial infarction, SIDS = sudden infant death syndrome, GSW= gun shot wound.

and 1 mM $MgCl_2$; pH 7.4) for two 15 minute periods at room temperature, and then transferred to fresh buffer containing [^{3}H]-SCH23390 for a 60 min incubation at room temperature. Six concentrations of [^{3}H]-SCH23390 (0.26, 0.39, 0.64, 0.96, 1.6, and 2.8 nM) were used. Mianserin (1 μM), a 5-HT_2 and 5-HT_{1C} receptor antagonist, was added to the incubation buffer to block binding of the radioligand to these sites. At each radioligand concentration, sections adjacent to those used to define total binding were incubated with [^{3}H]-SCH23390 in the presence of 10 μM *cis*-flupenthixol to estimate nonspecific binding. After incubation with [^{3}H]-SCH23390, all slides were washed in ice cold assay buffer for three 10-minute sessions, and then rinsed once in ice cold distilled water for two minutes. The tissue sections were allowed to dry overnight and apposed to Kodak X-OMAT film with polymer tritium-calibrated standards (Amersham; Arlington Heights, IL). To facilitate comparisons among subjects, the slide-mounted sections were arranged on film such that, for each radioligand concentration, one section of total or nonspecific binding from each subject was placed on a single large sheet of film. Film exposure proceeded for two to six months, depending on the concentration of [^{3}H]-SCH23390 used. The films were developed with Kodak D19 developer and GBX fixer, and the D_1 receptor density in defined regions was determined by densitometry using the MCID M4 software package (Imaging Research; St. Catherines, Ontario). The calibrated polymer standards were used to quantify the amount of radioligand bound, expressed as *n*Ci/mg tissue. For the determination of total binding, data from duplicate slides (representing adjacent sections) were averaged to arrive at a single value for each subject at each radioligand concentration.

Curvefitting of saturation binding isotherms was performed by nonlinear regression using Prism (Graphpad, San Diego, CA). ANOVA was used for comparison of binding parameters among age groups (SPSS:SYSTAT, Chicago, IL). Linear regression analysis with INSTAT (GraphPad, San Diego, CA) was conducted to examine the relation between age (as a quantitative variable) and D_1 receptor binding. The alpha level was set to 0.05 for all statistical procedures.

RESULTS

Autoradiography: D_1-Like Receptor Density in Human Brain

The saturation analysis of D_1 receptor sites in the caudate and putamen using [^{3}H]-SCH23390 showed that non specific binding increased linearly over the range of radioligand concentrations employed, and, at the highest radioligand concentration, 2.8 nM, accounted for 26% and 24% of the total binding in the caudate and putamen, respectively. Figure 1 displays autoradiograms of brain sections from representative subjects within the three age groups (see Table 1 for a history of the subjects used in the study).

A number of analyses were conducted to evaluate the relation between age and D_1 receptor density and affinity. First, nonlinear regression analysis was used to construct individual saturation isotherms for each subject; this was chosen as the primary analysis method because it made use of all of the data collected for each subject and provided independent estimates of the two parameters of interest, receptor density (B_{max}) and affinity (K_D). Summary data generated with this method are

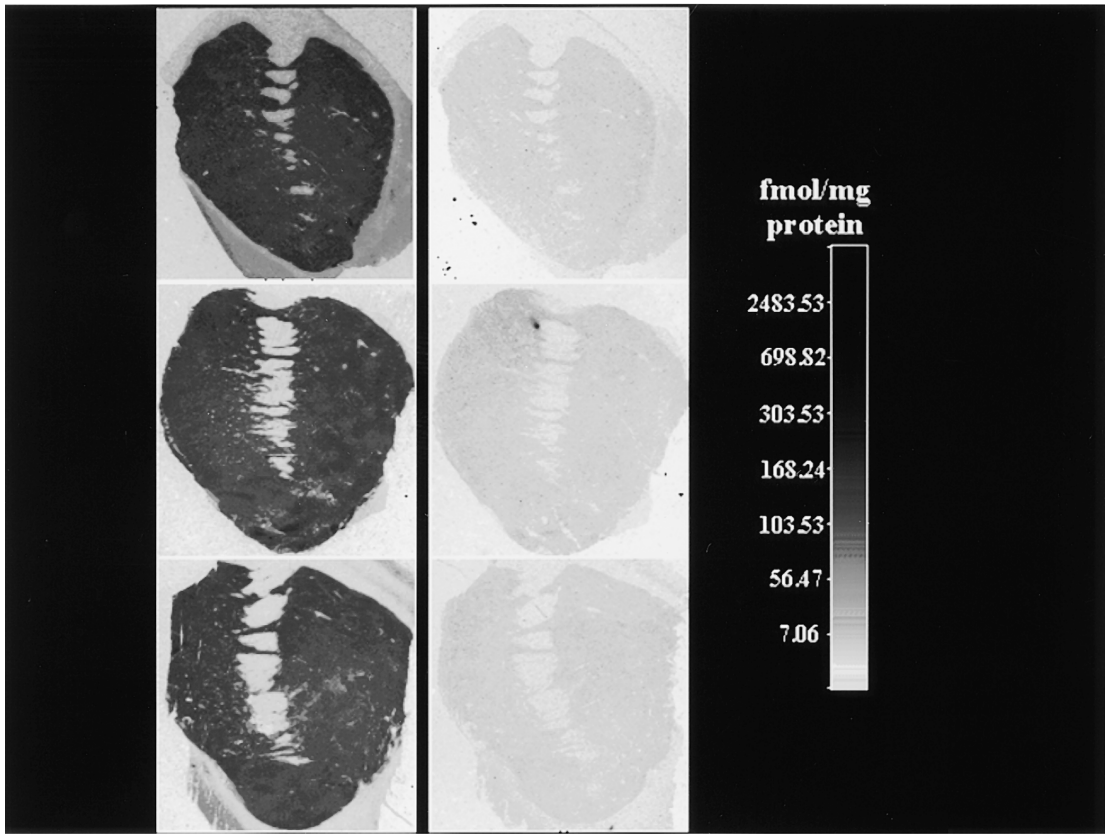

Figure 1. Autoradiogram of D_1 receptor-labeled sites ([^{3}H]-SCH23390 binding) in the caudate and putamen of human brain. These film images were obtained by incubation of brain sections with 0.96 nM of the D_1 receptor antagonist [^{3}H]-SCH23390. The column to the left represents total binding, whereas the column to the right reflects nonspecific binding (defined by 10 μM *cis*-flupenthixol). The top, middle, and bottom rows represent radioligand binding observed in a representative subject from the infant, adolescent, and adult subject groups, respectively. The calibration bar represents [^{3}H]-SCH23390 binding in fmol/mg protein.

presented in Table 2. The mean goodness of fit, indexed by r^2, ranged from 0.89 to 0.95 for the three groups. While these fits are less than ideal, it is likely that some portion of the discrepancy between the expected values and the observed data points represents variation associated with combining data from multiple sheets of film to construct each individual curve. An additional source of imprecision can be attributed to the fractional occupancy of D_1 receptors achieved in our study. Maximal fractional occupancy was estimated based on the highest radioligand concentration employed (2.8 nM) and the obtained dissociation constants (1.1–2.5 nM) where $f = C/(C + Kd)$. Maximal fractional occupancy ranged from 53–72%; thus, significant extrapolation was required to obtain Bmax values.

Differences in saturation binding parameters among age groups were evaluated with a series of univariate between-subjects ANOVAs, with a separate ANOVA performed for each brain region and dependent measure (K_D and B_{max}). There were no reliable differences in D_1 receptor affinity (K_D) among the three age groups [$F(2,12) = 1.50$, $p = .260$ for caudate; $F(2, 12) = 2.13$, $p = .161$ for putamen].

In contrast, D_1 receptor density (B_{max}) in putamen varied significantly among the three age groups [$F(2,12) = 4.05$, $p = .03$]. This effect could be attributed to a significant difference in B_{max} between adults and infants ($p < .05$ by Tukey-Kramer multiple comparison procedure). There also was a trend for the D_1 receptor density of adolescents to differ from that of adults ($p < .06$). There were no significant differences in D_1 receptor density among age groups in caudate [$F(2,12) = 0.324$, $p = .729$)].

Our second method of data analysis focused on an examination of the amount of specific binding obtained at selected radioligand concentrations. This method capitalized on the fact that, for each radioligand concentration, sections from all subjects were apposed to a single sheet of film, eliminating across-film sources of variance that were present when constructing saturation isotherms. Our analysis focused on the highest and lowest radioligand concentrations, 0.26 and 2.8 nM (see

Table 2. Summary of saturation isotherms of D_1 receptor-labeled sites ([^{3}H]-SCH23390 binding) in the caudate and putamen of human brain

	Caudate Nucleus			Putamen		
Age groups	**B_{max} (fmol/mg protein)**	**K_D (nM)**	**r^2**	**B_{max} (fmol/mg protein)**	**K_D (nM)**	**r^2**
Infant (n = 5)	1188 ± 281	1.6 ± 0.4	0.91 ± 0.03	1540 ± 233[a]	2.5 ± 0.3	0.95 ± 0.01
Adolescent (n = 6)	984 ± 108	1.1 ± 0.1	0.88 ± 0.02	1210 ± 101[b]	1.9 ± 0.2	0.91 ± 0.02
Adult (n = 4)	1030 ± 168	2.1 ± 0.6	0.94 ± 0.01	875 ± 120	1.9 ± 0.2	0.89 ± 0.02

Slide-mounted brain sections were incubated with varying concentrations of the D_1 receptor antagonist [^{3}H]-SCH23390. *Cis*-flupenthixol, 10 μM, was used to define nonspecific binding. For each subject, specific binding data were analyzed by nonlinear regression with a rectangular hyperbola to provide estimates of receptor density (B_{max}) and affinity (K_D). The data presented in the table represent the mean and standard errors of these parameter estimates. The r^2 values provide a goodness of fit measure of the nonlinear regression. The fractional occupancy of D_1 receptors achieved at the highest radioligand concentration employed, 2.8 nM, ranged from 53–72% based on the equation $f = C/(Ci + K_d)$.

[a] $p < .05$, [b] $p < .10$ compared to adults by Tukey-Kramer multiple comparison procedure conducted following significant omnibus ANOVA.

Table 3). The specific binding values obtained for each subject were log transformed and subjected to a 2 × 3 mixed model ANOVA, with age (three levels) as the between subjects factor, and radioligand concentration (two levels) as the within-subjects factor. Results from caudate and putamen were analyzed separately. The factorial analysis of log-transformed data was used to provide information about whether any differences in specific binding observed among age groups were likely due to differences in receptor affinity or in B_{max}. An age effect due primarily to a change in B_{max} would produce a constant proportional difference in specific binding among ages, regardless of the radioligand concentration. In the context of factorial ANOVA, log-transformed specific binding data would display a main effect of age and an absence of a statistical interaction between age and radioligand concentration (i.e., additivity of factor effects). Conversely, if differences observed in specific binding among age groups represented, at least partly, an alteration in receptor affinity, then the differences in log-transformed specific binding among age groups would vary at the two radioligand concentrations, with the result that a statistical interaction between age and radioligand concentration would be observed.

Our analysis of putamen data confirmed a significant effect of both age [$F(2,12) = 4.13$, $p = .042$] and radioligand concentration [$F(1,12) = 1477.87$, $p < .001$], and no interaction between the two factors [$F(2,12) = 0.716$, $p = .508$], a pattern of results consistent with an age-induced alteration in B_{max} rather than K_D. Comparisons between groups with the Tukey-Kramer procedure revealed trends for differences between adults and infants ($p < .06$) and adolescents and adults ($p = .07$).

An examination of the group means in the caudate displayed a trend similar to that in the putamen, although the age group differences did not achieve statistical significance [$F(2,12) = 1.95$, $p = .184$]. There was an expected main effect of radioligand concentration [$F(1,12) = 1267.1$, $p < .001$]. There was no interaction between age and radioligand concentration [$F(2,12) = 0.37$, $p = .70$].

Table 3. Receptor densities of D_1-labeled sites in caudate and putamen of human brain

		Specific binding of [^{3}H]-SCH23390 (fmol/mg protein)		
Brain region	**Conc (nM)**	**Infant (n = 4)**	**Adolescent (n = 6)**	**Adult (n = 5)**
Caudate nucleus	0.26	130 ± 10	118 ± 11	98 ± 24
	2.8	676 ± 69	619 ± 54	488 ± 52
Putamen	0.26	146 ± 11[a]	136 ± 9[a]	109 ± 12
	2.8	746 ± 82[a]	662 ± 46[a]	472 ± 58

Values represent means and their standard errors for [^{3}H]-SCH23390-labeled D_1 receptor specific binding at two selected radioligand concentrations in slide-mounted sections of caudate and putamen. A separate univariate ANOVA was conducted for each brain region and radioligand concentration.

[a] $p < .10$ compared to adults by Tukey-Kramer multiple comparison procedure conducted following significant omnibus ANOVA.

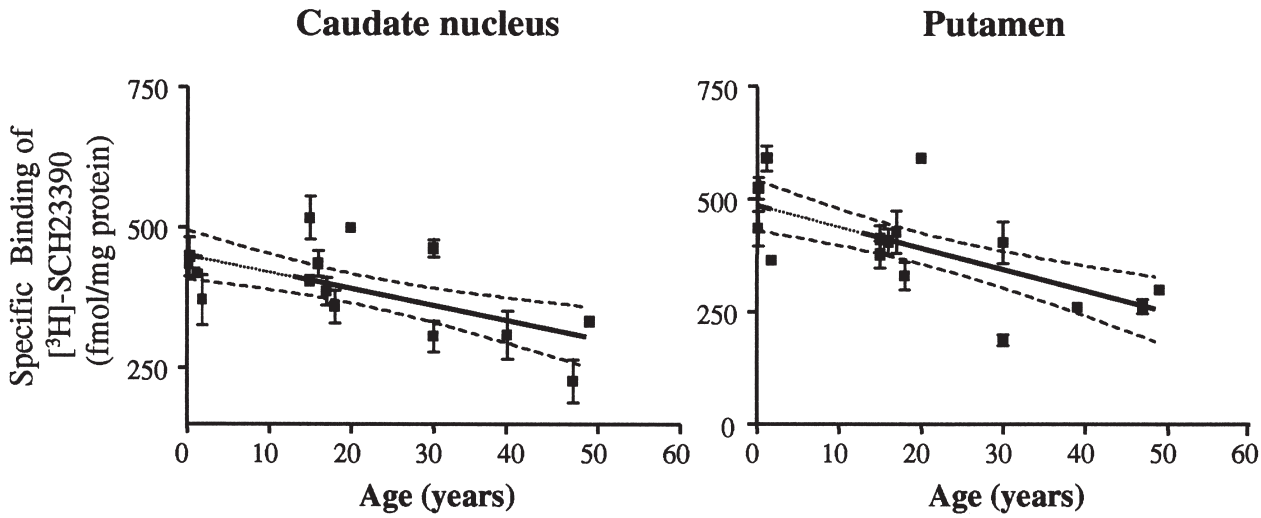

Figure 2. Correlation of age versus D_1 receptor density in the caudate nucleus and putamen. The results represent a correlation of age and specific binding of [^{3}H]-SCH23390 in slide-mounted sections of the caudate nucleus and putamen region of all the subjects tested. The abscissa represents increasing age in years while the ordinate represents D_1 receptor specific binding obtained with 0.96 nM of the radioligand, expressed in fmol/mg protein. The D_1 receptor fractional occupancy at 0.96 nM of [^{3}H]-SCH23390 is estimated to be 32%.The best fit line was determined by a least squares linear regression analysis. The dashed lines represent the limits of the 95% confidence interval of the regression line.

Finally, we performed linear regression analysis of specific binding obtained at each concentration as a function of age. This analysis allowed use of age as a quantitative rather than categorical variable. Figure 2 displays the results of the analysis obtained at a radioligand concentration of 0.96 nM. In both caudate nucleus and putamen, there was a significant, albeit modest, negative correlation between age and specific binding ($r = -0.61$, $p < .05$ in caudate; $r = -0.66$, $p < .05$ in putamen). Similar results were obtained with linear regression analysis of data obtained at other radioligand concentrations (data not shown).

DISCUSSION

The goal of this study was to examine the development of the dopamine D_1 receptor in normal human brain. We focused on the caudate and putamen due to the high density of D_1 receptors in these regions (Dawson et al. 1986), as well as the importance of these areas in the etiology, pathology, and/or treatment of both schizophrenia and Parkinson's disease (Graybiel 1997). Our results, obtained using several complementary methods of analysis, provide strong evidence for developmental regulation of human D_1 receptor number, but not affinity. The differences in receptor density among those age groups surveyed in the present study were most apparent in the putamen, although a similar trend was observed in the caudate nucleus.

Our demonstration of an age-related variation in dopamine receptor density is consistent with prior studies in several species of mammalian brain, including rodents, monkeys, and humans (Rinne et al. 1990; Seeman et al. 1987; Palacios et al. 1988; De Keyser et al. 1990; Schambra et al. 1994; Lidow 1995; Teicher et al. 1995; Boyson and Adams 1997). More importantly, our results from adolescent samples provide new information about dopamine D_1 receptor density during a time period that has been markedly under-represented in past studies. The present findings thus extend prior studies in this area, and provide a means to evaluate and refine current notions about dopamine receptor development.

A comparison of our results to those obtained by Seeman et al. (1987) is warranted. Seeman et al. (1987) raised the possibility of a multi-component function relating age and D_1 receptor density. The initial component was revealed by a very sharp rise in D_1 receptor density from infancy to late childhood. In contrast, receptor levels in young adult samples in Seeman's study were markedly lower, implying a precipitous decline during adolescence. In our own study, we did not have access to tissue from children, thus we could not confirm the presence of a transient, explosive increase in dopamine receptor density in this age group. Our finding that adolescent receptor density tended to be somewhat lower than that of infants does rule out the possibility, however, that large D_1 receptor density increases are initiated or maintained during adolescence. Moreover, the receptor densities of adolescents in our study

did not display large variation among subjects. This latter finding would be unexpected if adolescence represented the steep descending limb of a large prior peak in D_1 receptor density. Our data thus imply that any precipitous childhood over-expression of D_1 receptors is time-limited and does not extend appreciably into adolescence, or alternately, does not occur at all.

The present results, taken together with past findings, underscore the need for additional studies that are powered sufficiently to define more clearly the temporal expression of dopamine receptors in the period from early postnatal life through adolescence in humans. Synaptic overproduction and pruning have been envisioned as the primary forces that shape brain maturation during this critical time period. Evidence of postnatal overproduction and subsequent pruning of neurotransmitter receptors in primates can be found in a series of elegant studies in which dopaminergic, serotonergic, noradrenergic and adrenergic systems in macaque cortex have a postnatal increase and eventual decline in receptor density prior to puberty (Rakic et al. 1986; Lidow et al. 1991; Lidow and Rakic 1992). Studies in rodent striatum likewise provide evidence for overproduction and pruning of D_1 receptors (Teicher et al. 1995).

The issue of synaptic over-production followed by pruning may have relevance to the development of schizophrenia. Feinberg and others have postulated that these events, or possibly alterations in these events, may play a large role in the etiology and actual onset of schizophrenia (Feinberg 1982; Keshavan et al. 1994; Teicher et al. 1995). Early perturbations of dopamine receptors may have relevance also to the expression of certain neurological disorders such as Tourette's syndrome, that typically emerge during middle to late childhood (Leckman et al. 1997).

In addition to its relevance to developmental hypotheses concerning neurological and neuropsychiatric disorders (Seeman et al. 1987; Carlsson and Carlsson 1990; Laruelle et al. 1996), our work bears on the role of dopamine receptors in normal aging. Our findings are consistent with a continual age-related decline in D_1 receptor density throughout adulthood. This effect can be seen clearly in the correlational analysis presented in Figure 2. Several markers of dopaminergic neurons display a gradual increase throughout adulthood (Bannon et al. 1992; Palmer and DeKosky 1993), much like that observed for D_1 receptors. The loss of dopamine D_1 receptors does not appear to be a trivial consequence of the loss of dopamine neurons, however, because this receptor subtype is not expressed by dopamine neurons. Rather, the D_1 receptor is found exclusively on postsynaptic target neurons. For example, D_1 receptors are localized on GABAergic neurons that project from the basal ganglia to the substantia nigra and globus pallidus (Gerfen 1992, 1995). It is possible that the loss of dopamine neurons causes adaptive changes in target neurons, thereby resulting in decreased expression of D_1 receptors. Alternatively, the decrease in D_1 receptors may reflect concomitant loss of target neurons (e.g., possible age-related decreases in GABAergic neurons). This latter possibility is supported by a prior study in rodents (Zhang and Roth 1997). This study demonstrated that the total number of striatal neurons expressing D_1 receptor mRNA was decreased in aged brain, although there was no reduction in the D_1 mRNA content in individual neurons.

One limitation of the present study, and all prior studies in this area, is the inability to distinguish between D_1 and D_5 receptors. The radioligand employed, [^{3}H]-SCH23390, binds with similar affinity to both D_1 and D_5 receptors (Weinshank et al. 1991; Sunahara et al. 1991). Available evidence in primate brain suggests a much lower expression level of D_5 vs. D_1 receptors in those striatal regions examined in the present study (Bergson et al. 1995a, b; Choi et al. 1995; Lidow et al. 1998), suggesting that the binding we observed represented primarily D_1 receptors. It is possible, however, that the pattern of development of D_1 and D_5 receptors differs; addressing this issue must await the availability of subtype-selective ligands.

It should be noted that two other factors, gender and postmortem interval, might have affected these results. While the limited sample size in this study made it impossible to evaluate the influence of these factors directly, previous studies do provide some relevant data. Pohjalainen et al. (1998) did not find a statistically reliable gender difference in striatal D_2 receptor expression when measured *in vivo*, although there was a trend toward a more rapid age-related decline in women. We previously evaluated the effects of postmortem interval by performing controlled experiments in rats under conditions that simulated the postmortem cooling of human brain. After a 24-hour postmortem interval, only a 6% decrease in [^{3}H]-SCH23390 binding to D_1 receptors occurred in caudate, and this small decrease was restricted to the first six hours after death (Gilmore et al. 1993). Notably, all of the samples in the present study had postmortem intervals from 9 to 25 hrs. These previous studies suggest that any effects of gender or postmortem interval are modest and unlikely to impact our results.

In summary, the present study found no age-related changes in affinity of [^{3}H]-SCH23390 for the D_1 receptor in the caudate and putamen among the three age groups, whereas an age-dependent decreased receptor density that continues throughout the human life span was apparent. The present data underscore the need to differentiate between the normal pruning that occurs in the development of the human nervous system, and the aging-related decline in function. Understanding the involved mechanisms will be useful in elucidating the

role of changes in dopamine neurotransmission in the etiology and therapy of diseases like schizophrenia and Parkinson's disease.

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