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Chronic haloperidol alters dopamine receptors: effects of cocaine exposure during the preweaning period

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Abstract

The effect of cocaine exposure during the preweaning period on the function of the central dopaminergic systems was determined in adult rats. The present study investigated the alterations in dopamine receptors in 93-day-old male and female rats treated with cocaine (50 mg kg^{-1} day^{-1}), 1-[2-[bis(4-fluorophenyl)methoxyl]-4-[3-phenylpropyl]piperazine (GBR 12909) (50 mg kg^{-1} every other day) or water during postnatal days 11–20. Haloperidol (2 mg kg^{-1} day) or saline was injected during postnatal days 76–90 and the rats were killed on postnatal day 93. Quantitative receptor autoradiography with [^{3}H]*R*-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine ([^{3}H]SCH 23390) for the dopamine D_1 receptor and [^{3}*H*]raclopride for the dopamine D_2 receptor was carried out. The results show that haloperidol increased [^{3}H]raclopride binding in many forebrain regions. Preweaning cocaine treatment in males increased the area showing this effect. Males generally were more responsive to haloperidol than females. However, in GBR 12909-treated females, raclopride binding showed widespread increases following haloperidol injection. For SCH 23390 binding, most regions showed a significant interaction between haloperidol, sex and preweaning treatment group. This was due primarily to the GBR 12909-treated males, which showed elevated basal dopamine D_1 receptor binding levels and a haloperidol-induced reduction in dopamine D_1 receptor binding in most regions evaluated. These data suggest that inhibition of the dopamine transporter during ontogeny produces long-term alterations in dopamine receptor regulation but that selective inhibitors of the dopamine transporter produced greater effects than cocaine on both raclopride and SCH 23390 binding following chronic haloperidol injection.

Keywords: Cocaine; GBR 12909; Dopamine D_1 receptor; Dopamine D_2 receptor; Haloperidol; Basal ganglion

1. Introduction

The human brain undergoes development throughout the prenatal period of gestation as well as during a substantial portion of childhood. Since the timing of ontogenic events in the brain relative to birth is different in human and rodent species, rodent models of human prenatal development must include events occurring during the postnatal period. Although the exact timing of events relative to birth is not known, the state of maturation of the human brain at 19–20 weeks gestation is roughly equivalent with that of the rat on the day of birth (Bayer et al., 1993). Therefore, the postnatal period in the rat approximates the later half of prenatal development in humans. During the postnatal period of the rat, the dopamine system, in particular, undergoes extensive maturation since substantial changes in dopamine receptors, second messenger systems, transporters, enzymes, transmitter content and electrophysiologic responses have been characterized (Murrin and Zeng, 1986, 1989; Rao et al., 1991; Teicher et al., 1991; Srivastava et al., 1992; Wang and Pitts, 1994, 1995). Previous studies have shown that exposure to cocaine during PND 11–20 decreases the behavioral responses to a selective dopamine D_1 receptor agonist (Dow-Edwards and Busidan in revision), decreased the concentration of preprodynorphin mRNA in nucleus accumbens (Dow-Edwards and Hurd, 1998), and decreased the concentration of dopamine transporter mRNA in ventral mesencephalon (Dow-Edwards et al., 1997). Since these represent functional changes in the dopamine system, we hypothesized that preweaning cocaine may affect the neurochemical

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response to chronic dopamine blocking agents, such as haloperidol.

In order to compare cocaine's effects with those produced by selective inhibitors of the dopamine transporter, we gave another group of rats GBR 12909, a highly selective dopamine transporter inhibitor (Andersen, 1989; Heikkila and Manzino, 1984) and examined the response to haloperidol also. We were interested in determining (1) if cocaine and GBR 12909 can change the response of the dopamine receptors to haloperidol and (2) if both genders responded similarly.

2. Materials and methods

2.1. Animals

Sprague–Dawley rats (CD-COBS, Charles River, Wilmington, ME) were mated in our AAALAC-accredited animal facility and left undisturbed until day of birth. On day of birth (postnatal day 1) litters were culled to eight with equal numbers of males and females. Litters were assigned to receive cocaine HCl (Sigma, St. Louis, MO), GBR 12909-diHCl (Research Biochemicals International, Natick, MA) or vehicle (sterile water, Baxter, Deerfield, IL). Each group was injected with 50 mg/kg body weight cocaine-HCl, GBR 12909-diHCl or vehicle subcutaneously on PND 11 through 20 in a volume of microliters equal to five times their body weight in grams. Since the half-life of GBR 12909 is significantly longer than that of cocaine (Benfield et al., 1986; Ingwersen et al., 1993), GBR 12909 was administered on 'odd' days and the vehicle was administered on the 'even' days. Starting on postnatal day 75 all rats were injected with 2 mg/kg haloperidol (Schiapparelli Searle, Chicago, IL) or an equivalent volume of saline (Baxter, Deerfield, IL) intraperitoneally for 15 days. The rats were killed with a lethal dose of Na-pentobarbital on postnatal day 93, 3 days after last dose. The brains were removed and frozen. All brains were sectioned at 20 μm for quantitative dopamine D_1 and D_2 receptor autoradiogrphy. These studies were approved by Institutional Animal Care and Use Committee.

2.2. Dopamine D_1 receptor binding

Frozen slide-mounted sections were removed from −80°C storage and placed in 0°C storage 12 h prior to incubation in order to allow the tissue to slowly defrost. The sections were preincubated using a standard published protocol (Dawson et al., 1986) for 30 min at room temperature in 50 mM Tris–HCl (pH 7.4) containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and 6 mM ascorbic acid. The slides were then placed in the incubation medium for 45 min at room temperature containing the same solution as the preincubation medium plus 6 nM [^{3}H]*R*-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine ([^{3}H]SCH 23390) (NEN Research Products, Boston, MA. specific activity, 81.4 Ci/mmol). This concentration of radioligand was chosen because it represents approximately three times the dissociation constant (K_D) value (Dawson et al., 1986) resulting in approximately 75% receptor occupancy. Alternate sections were incubated in the same media, with the addition of (+)-butaclamol (10 μM) (Research Biochemicals International, Natick, MA) to produce autoradiographs representing nonspecific binding. Ketanserin (40 nM) (Research Biochemicals International, Natick, MA) was added to all incubation media to block serotonin receptors. The sections were then washed twice (5 min each) in ice-cold buffer, and the buffer salts were removed by a rapid dip in ice-cold distilled water. The sections were dried with a stream of cool dry air. Autoradiographs were generated by apposition of the slides to hyperfilm-^{3}H (Amersham, Sweden) in lead X-ray cassettes along with [^{3}H]microscale standards (Amersham, Sweden) for 10 days at which time the films were removed and developed.

2.3. Dopamine D_2 binding protocol

All solutions were the same concentration of salts as described above, except 8 nM [^{3}H]raclopride (Köhler and Radesäter, 1986) (NEN Research Products, Boston, MA. specific activity 85.6 Ci/mmol) replaced the SCH 23390. This concentration of radioligand was chosen because it represents approximately three times the dissociation constant (K_D) value (Mansour et al., 1990) resulting in approximately 75% receptor occupancy. Alternate sections were incubated in the same media, with the addition of (+)-butaclamol (10 μM) to produce autoradiograms representing nonspecific binding. The sections were then washed four times (3 min each) in ice cold buffer and the buffer salts were removed by a rapid dip in ice-cold distilled water. The sections were dried with a stream of

Fig. 1. Illustrations (adapted from Paxinos and Watson, 1986) of coronal sections of rat brain with the locations of regions of interest analyzed in this study. (A) Taken at 2.7 mm rostral to bregma indicating where the rostral accumbens (Acb-Rost) was analyzed. (B) Taken 1.6 mm rostral to bregma indicating the locations of accumbens core and shell (Acb-C and Acb-S) and olfactory tubercle (OT). Similar accumbal areas were assessed at 1.2 mm rostral to bregma (not shown). (C) Taken from 0.2 mm rostral to bregma at the level of the caudate nucleus. Superior medial and lateral (SM and SL) and inferior medial and lateral (IM and IL) quadrants were analyzed as well as the full caudate. (D) Taken 4.8 mm caudal to bregma at the level of the substantia nigra pars compacta and reticulata (SN-PC and SN-PR) and the ventral tegmental area (VTA).

cool dry air. Autoradiographs were generated by apposition of the slides to hyperfilm-^{3}H (Amersham) in lead X-ray cassettes along with [^{3}H]microscale standards (Amersham) for 3 weeks.

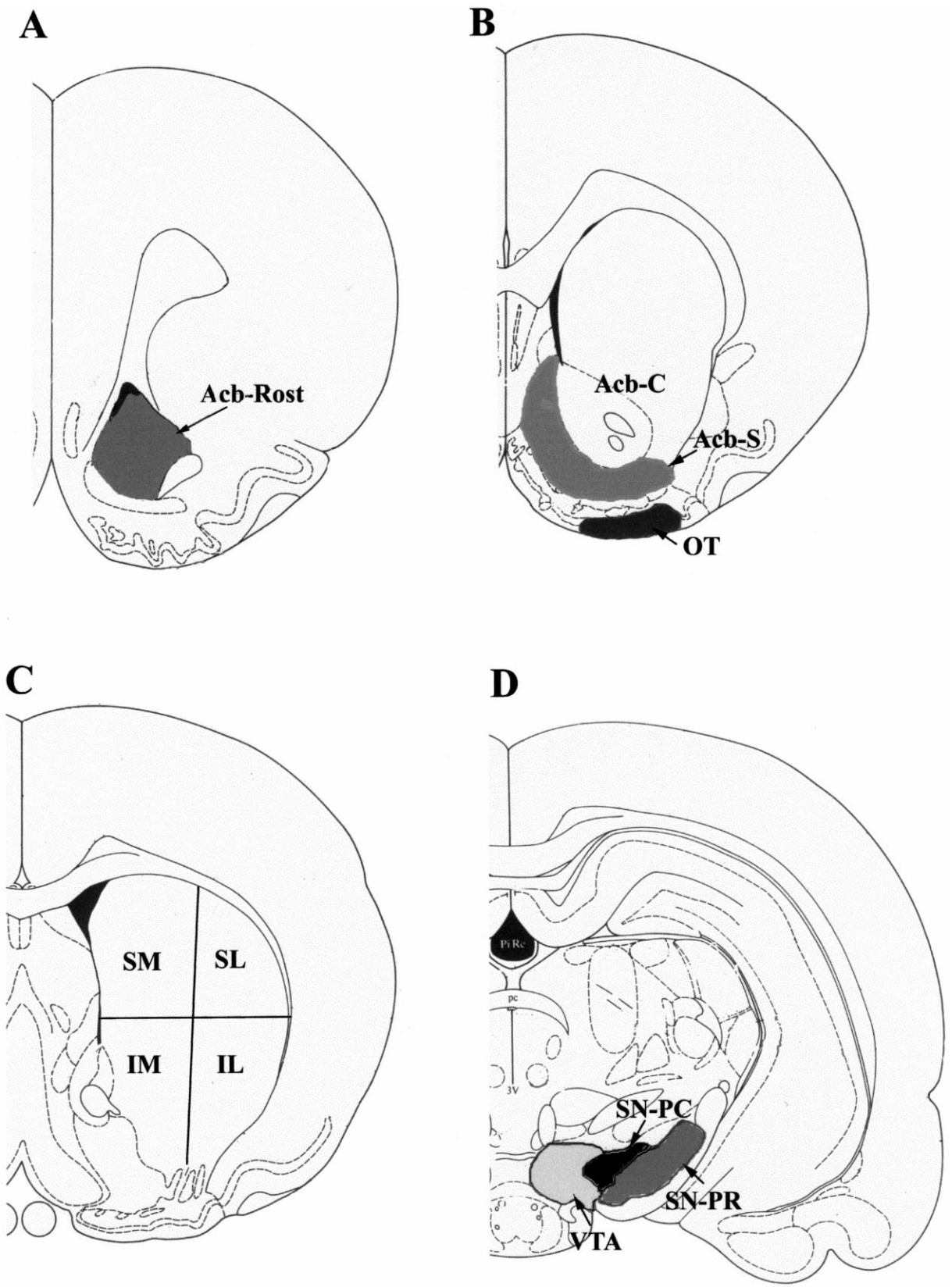

Table 1
Dopamine D2 receptor binding ([^{3}H]raclopride) binding in 93-day-old rats treated with saline and haloperidol: pretreatment with vehicle, cocaine and GBR12909 during postnatal 11–20 days

	Male			Female			2-way ANOVA treatment *P*-value	3-way ANOVA effect of HAL *P*-value
	Vehicle	Cocaine	GBR12909	Vehicle	Cocaine	GBR12909		
Caudate nucleus—whole								
Saline	88.9 ± 8.0	92.2 ± 6.8	104.6 ± 4.7	98.2 ± 8.6	99.0 ± 6.7	93.6 ± 4.0	0.714	0.000
Haloperidol	113.8 ± 6.7	117.7 ± 3.2	123.7 ± 5.0	121.3 ± 8.6	115.6 ± 4.6	129.1 ± 4.7	.0221	
P-value (*t*-test)[a]	0.032	0.005	0.016	0.083	0.064	0.000		
Caudate-sup-med								
Saline	77.1 ± 7.2	76.7 ± 5.9	89.6 ± 4.2	83.1 ± 7.4	81.9 ± 6.9	76.0 ± 3.4	0.838	0.000
Haloperidol	95.7 ± 6.9	100.8 ± 3.6	102.4 ± 4.0	104.4 ± 7.9	100.2 ± 4.2	109.4 ± 4.9	0.525	
P-value (*t*-test)	0.076	0.005	0.047	0.073	0.043	0.000		
Caudate-sup-lat								
Saline	96.6 ± 9.0	103.1 ± 7.6	117.9 ± 6.9	105.9 ± 9.9	107.0 ± 7.4	100.8 ± 4.8	0.599	0.000
Haloperidol	119.5 ± 7.7	126.6 ± 4.2	135.6 ± 5.5	130.7 ± 10.2	125.1 ± 6.2	141.2 ± 6.3	0.136	
P-value (*t*-test)	0.075	0.019	0.067	0.108	0.085	0.000		
Caudate-inf-med								
Saline	64.7 ± 5.8	63.6 ± 5.0	75.2 ± 2.6	74.8 ± 6.6	73.7 ± 6.0	69.5 ± 3.7	0.763	0.000
Haloperidol	86.0 ± 4.2	88.8 ± 4.1	95.1 ± 7.0	90.6 ± 7.3	85.7 ± 3.9	96.6 ± 2.4	.0221	
P-value (*t*-test)	0.010	0.002	0.020	0.134	0.121	0.000		
Caudate-inf-lat								
Saline	115.1 ± 9.2	122.8 ± 8.8	131.8 ± 5.1	128.9 ± 10.8	130.0 ± 7.0	128.5 ± 5.1	0.603	0.000
Haloperidol	149.5 ± 8.5	152.9 ± 5.4	161.0 ± 8.1	157.1 ± 10.1	149.9 ± 4.8	165.7 ± 7.7	0.255	
P-value (*t*-test)	0.017	0.013	0.010	0.083	0.037	0.000		
Nucleus accumbens—Core-1.6[b]								
Saline	59.5 ± 7.3	61.3 ± 3.5	59.3 ± 5.2	70.0 ± 5.4	65.0 ± 3.9	66.9 ± 4.8	0.936	0.000
Haloperidol	82.1 ± 3.5	81.4 ± 5.0	93.2 ± 3.8	86.4 ± 7.3	78.7 ± 4.1	88.6 ± 2.2	0.101	
P-value (*t*-test)	0.012	0.009	0.000	0.096	0.034	0.000		
Nucleus accumbens—shell-1.6								
Saline	47.2 ± 5.7	48.3 ± 2.7	52.7 ± 2.8	54.7 ± 4.1	51.2 ± 3.4	55.5 ± 2.7	0.539	0.000
Haloperidol	64.9 ± 2.4	64.8 ± 5.8	76.3 ± 4.0	65.7 ± 6.8	60.5 ± 3.2	67.7 ± 2.5	0.127	
P-value (*t*-test)	0.010	0.031	0.001	0.193	0.070	0.007		
Nucleus accumbens—rostral								
Saline	80.2 ± 8.7	79.9 ± 3.0	94.0 ± 2.0	92.4 ± 6.0	91.8 ± 5.9	93.4 ± 5.3	0.294	0.000
Haloperidol	113.8 ± 4.7	110.9 ± 5.1	118.0 ± 7.4	116.9 ± 5.5	113.1 ± 3.7	125.3 ± 2.0	0.176	
P-value (*t*-test)	0.004	0.000	0.009	0.011	0.010	0.000		
Olfactory tubercle								
Saline	61.7 ± 6.8	61.9 ± 3.3	70.9 ± 2.0	71.9 ± 5.5	68.7 ± 4.1	70.9 ± 2.7	0.417	0.000
Haloperidol	76.6 ± 2.9	76.1 ± 4.4	82.2 ± 9.2	79.2 ± 5.5	75.0 ± 2.1	81.4 ± 3.9	0.489	
P-value (*t*-test)	0.054	0.024	0.253	0.362	0.199	0.045		
Substantia nigra pars compacta								
Saline	21.4 ± 1.6	21.4 ± 0.4	23.8 ± 0.8	20.5 ± 2.4	20.6 ± 0.8	22.0 ± 1.1	0.202	0.126 S
Haloperidol	22.0 ± 1.1	24.9 ± 1.5	24.5 ± 2.0	22.3 ± 1.4	22.1 ± 0.5	20.9 ± 0.7	0.594	
P-value (*t*-test)	0.746	0.046	0.712	0.497	0.128	0.418		
Substantia nigra pars reticulata								
Saline	16.7 ± 0.9	16.0 ± 0.6	16.9 ± 0.4	16.9 ± 0.8	14.5 ± 0.7	15.1 ± 0.7	0.131	0.027
Haloperidol	16.7 ± 1.1	19.3 ± 0.7	17.9 ± 1.9	17.5 ± 1.3	17.3 ± 1.3	15.2 ± 0.5	0.358	
P-value (*t*-test)	0.982	0.004	0.586	0.715	0.068	0.890		
Ventral tegmental area								
Saline	20.0 ± 1.6	18.8 ± 0.9	19.7 ± 1.7	19.9 ± 1.1	19.8 ± 1.0	20.3 ± 0.6	0.842	0.002
Haloperidol	22.9 ± 1.7	23.0 ± 1.4	24.6 ± 3.5	21.5 ± 1.7	23.5 ± 0.7	20.9 ± 1.1	0.850	
P-value (*t*-test)	0.233	0.031	0.205	0.426	0.012	0.615		

2.4. Image analysis

Analysis of the autoradiographs was accomplished using computer-assisted densitometric analysis (M1 Imaging System, Imaging Research) with the investigator unaware of the experimental treatments of the animals. Optical densities were converted into femtomoles per milligram of tissue (fmol/mg tissue) by referencing the tritium-labeled microscales. The mean densities for the regions of interest were measured from at least two sections per region per rat. Structures were identified based on the atlas of Paxinos and Watson (1986) in adjacent thionin stained sections (see Fig. 1). The caudate putamen and nucleus accumbens were subdivided into quadrants and core/shell, respectively, and assessed as multiple subregions of these structures.

2.5. Statistical analysis

All data were analyzed on a region by region basis using a 3-way analysis of variance (ANOVA) to compare preweaning treatment groups (vehicle, cocaine and GBR 12909), gender (male and female) and haloperidol condition (saline and haloperidol) using SYSTAT. A 2-way ANOVA was used to compare the differences in saline or haloperidol injected groups (treatment by gender) separately. Post-hoc tests were carried out to determine if the cocaine group was significantly different from the control when treatment was significant. Two-tailed *t*-test was used to determine the significance of differences between saline and haloperidol injections within the same gender/preweaning treatment groups. The minimum accepted level of significance in the ANOVA and post hoc test was $P \leq 0.05$. In the *t*-test, significance was set at $P \leq 0.01$ to adjust for multiple comparisons.

3. Results

3.1. Raclopride binding

The effects of haloperidol on the upregulation of dopamine D_2 receptors of adult rats treated with cocaine or GBR 12909 during postnatal days 11–20 are summarized in Table 1. Haloperidol significantly increased raclopride binding in 11 of 12 areas examined by 3-way ANOVA ($P < 0.05$). Only the substantia nigra pars compacta showed no significant haloperidol effect. However, this region showed a significant gender effect with females having lower binding levels than males. In general, the substantia nigra pars compacta and reticulata and the ventral tegmental area, the midbrain cell body regions, showed weaker haloperidol effects than all the dopamine rich terminal regions.

Raclopride binding was significantly increased in vehicle-treated males in 3 of 12 regions by haloperidol injection ($P \leq 0.01$). In cocaine-treated males, the effect of haloperidol was widespread with 7 of 12 areas showing significant increases. Preweaning GBR 12909-treated males showed increased raclopride binding in 4 of 12 regions. In the vehicle-treated females, no region showed significantly affected raclopride binding following haloperidol injection and the cocaine-treated females showed only 1 region altered by haloperidol. On the other hand, in the females treated with GBR 12909, raclopride binding was increased in 8 of 12 regions ($P \leq 0.01$). Not only were the percentage increases greater in the GBR 12909-treated females receiving haloperidol but the number of affected areas was the largest of all gender/treatment groupings.

3.2. SCH 23390 binding

SCH 23390 binding in rats treated with cocaine, GBR 12909 or vehicle during postnatal days 11–20 and then injected with saline during postnatal days 75–90 indicates significant effects of preweaning treatments in all brain regions analyzed ($P \leq 0.05$, 2-way ANOVA, Table 2). There were also significant interactions between sex and preweaning treatment group in all regions ($P \leq 0.001$, 2-way ANOVA. Table 2, m) with binding levels in females being greater than in males. Within each gender, post-hoc analysis indicated that a significant difference between the preweaning vehicle-treated males and the males treated with GBR 12909 was seen in each region analyzed ($P \leq 0.05$, Dunnett, Table 2, a). There were no other differences between preweaning treatment groups and the same-gender vehicle-treated controls. Within the cocaine-treated groups injected with saline, however, significant differences in binding were found between the males and females in 5 of 12 regions including most of the nucleus accumbens, the olfactory tubercle and the inferior-lateral caudate (Table 2, c). Within the GBR

Notes to Table 1:
The values are the means ± S.E.M. ($n = 6–8$/cell) in fmol/mg tissue.
Statistical comparisons were made by three-way analysis of variance (ANOVA), two-way ANOVA and *t*-test.
There are no significant differences by two-way ANOVA (treatment by sex) in saline and haloperidol groups.
P-values of treatment main effect are indicated in 2-way column.
Significant main effect of haloperidol, the *P*-values are indicated in 3-way ANOVA column.
S: Significant difference between male and female.
[a] *P*-value (*t*-test) between saline and haloperidol groups which had same preweaning treatment.
[b] Indicates the section level based on the atlas of Paxinos and Watson (1986).

Table 2
Dopamine D1 receptor binding ([^{3}H]-SCH 23390) in 93-day-old rats treated with saline and haloperidol: pretreatment with vehicle, cocaine and GBR12909 during postnatal 11–20 days

	Male			Female			2-way ANOVA treatment effect	3-way ANOVA *P*-value
	Vehicle	Cocaine	GBR12909	Vehicle	Cocaine	GBR12909	*P*-value[b]	*x*
Caudate—putamen—whole								
Saline	199.8 ± 11.8	208.6 ± 5.8	294.4 ± 15.1[a]	225.8 ± 16.3	250.8 ± 20.3	217.2 ± 13.7	0.020 mg	GF
Haloperidol	227.9 ± 11.8	233.1 ± 14.9	227.9 ± 12.3	233.8 ± 7.2	231.8 ± 12.4	244.9 ± 21.9	0.919	
P-value (*t*-test)[d]	0.114	0.152	0.005	0.648	0.440	0.326		
Caudate-sup-med								
Saline	195.5 ± 11.9	204.5 ± 6.8	292.3 ± 16.6[a]	216.7 ± 14.3	238.9 ± 20.5	207.2 ± 12.8	0.017 mg	GF
Haloperidol	221.7 ± 11.2	228.3 ± 15.3	218.3 ± 11.3	229.2 ± 7.8	227.3 ± 13.6	233.5 ± 23.3	0.986	
P-value (*t*-test)	0.130	0.181	0.003	0.440	0.645	0.366		
Caudate-sup-lat								
Saline	188.6 ± 11.9	202.1 ± 16.1	287.4 ± 17.1[a]	217.9 ± 18.4	243.1 ± 21.6	203.8 ± 14.3	0.037 mg	GF
Haloperidol	208.6 ± 12.7	219.9 ± 14.1	216.1 ± 9.3	217.2 ± 8.1	221.2 ± 12.6	236.9 ± 21.9	0.614	
P-value (*t*-test)	0.272	0.268	0.003	0.972	0.436	0.249		
Caudate-inf-med								
Saline	172.4 ± 9.7	181.2 ± 6.8	264.3 ± 16.9[a]	195.5 ± 13.4	219.2 ± 17.6	187.3 ± 12.4	0.013 mg	GF
Haloperidol	209.2 ± 8.5	209.3 ± 15.3	204.3 ± 15.5	207.7 ± 6.1	202.3 ± 7.7	210.8 ± 19.0	0.977	
P-value (*t*-test)	0.013	0.118	0.002	0.403	0.397	0.339		
Caudate-inf-lat								
Saline	233.2 ± 13.4	239.5 ± 5.7	338.4 ± 15.6[a]	264.7 ± 20.1	291.3 ± 23.8	262.3 ± 15.3	0.014 mg	CG
Haloperidol	263.4 ± 14.1	266.5 ± 16.2	263.3 ± 15.5	267.6 ± 7.9	266.6 ± 13.9	285.5 ± 22.8	0.820	
P-value (*t*-test)	0.143	0.145	0.005	0.890	0.388	0.417		
Nucleus accumbens—Core-1.2[e]								
Saline	143.0 ± 7.0	150.7 ± 6.6	209.0 ± 10.4[a]	163.7 ± 9.4	180.5 ± 9.2	168.9 ± 11.0	0.001 gmc	CEF
Haloperidol	190.4 ± 5.3	164.8 ± 15.5	191.5 ± 14.3	186.6 ± 8.7	162.7 ± 4.4	161.1 ± 11.4	0.051	
P-value (*t*-test)	0.000	0.418	0.332	0.096	0.162	0.609		
Nucleus accumbens—Shell-1.2								
Saline	121.1 ± 7.4	131.3 ± 5.0	176.6 ± 7.2[a]	136.0 ± 7.5	153.2 ± 9.4	144.7 ± 8.3	0.001 mg	EF
Haloperidol	168.3 ± 6.6	144.3 ± 13.2	158.7 ± 8.4	158.6 ± 10.3	139.1 ± 4.3	131.5 ± 7.5	0.037	
P-value (*t*-test)	0.000	0.376	0.140	0.101	0.198	0.268		
Nucleus accumbens—Core-1.6								
Saline	191.0 ± 5.9	198.8 ± 7.2	267.4 ± 16.8[a]	209.0 ± 12.8	230.9 ± 8.8	215.6 ± 7.0	0.001 mgc	CEFG
Haloperidol	226.1 ± 13.2	203.2 ± 11.4	221.0 ± 16.3	220.7 ± 10.7	198.4 ± 6.2	222.7 ± 12.2	0.131	
P-value (*t*-test)	0.029	0.746	0.070	0.490	0.11	0.619		
Nucleus accumbens—Shell-1.6								
Saline	179.7 ± 6.1	189.5 ± 10.5	243.4 ± 14.5[a]	199.4 ± 14.1	220.3 ± 6.0	214.4 ± 12.1	0.003 mc	E
Haloperidol	212.5 ± 12.1	189.7 ± 13.7	202.9 ± 21.7	202.8 ± 11.3	184.0 ± 10.5	198.5 ± 12.7	0.325	
P-value (*t*-test)	0.030	0.991	0.146	0.854	0.011	0.384		
Nucleus accumbens—rostral								
Saline	262.6 ± 12.4	258.2 ± 6.1	360.4 ± 14.2[a]	286.3 ± 19.1	323.9 ± 23.4	290.0 ± 10.0	0.006 mcg	CG
Haloperidol	288.7 ± 10.9	285.0 ± 20.9	281.1 ± 20.3	284.7 ± 10.5	278.1 ± 13.5	309.6 ± 21.7	0.713	
P-value (*t*-test)	0.125	0.240	0.007	0.942	0.122	0.428		
Olfactory tubercle								
Saline	267.0 ± 14.9	262.8 ± 7.5	358.7 ± 10.5[a]	292.1 ± 21.1	329.8 ± 18.6	287.2 ± 11.8	0.002 mcg	G
Haloperidol	303.0 ± 17.9	304.5 ± 22.5	287.7 ± 14.5	302.8 ± 8.8	299.2 ± 9.4	321.4 ± 18.9	0.987	
P-value (*t*-test)	0.144	0.104	0.002	0.631	0.168	0.150		
Substantia nigra								
Saline	215.9 ± 14.0	231.9 ± 15.3	297.1 ± 9.5[a]	255.0 ± 17.6	275.8 ± 18.7	251.8 ± 12.0	0.046 mg	CFG
Haloperidol	246.7 ± 17.1	272.7 ± 5.9	271.3 ± 16.7	260.1 ± 5.9	252.6 ± 11.1	268.1 ± 16.5	0.460	
P-value (*t*-test)	0.254	0.024	0.209	0.791	0.313	0.441		

12909-treated groups, 11 of 12 regions examined showed differences between the genders including most of the caudate, accumbens, olfactory tubercle and substantia nigra (Table 2, g).

For the haloperidol-injected rats, the only region showing a main effect of preweaning treatment was the nucleus accumbens shell at 1.2 (Table 2). No region showed a significant gender difference ($P > 0.05$, 2-way ANOVA). Therefore, haloperidol effectively eliminated the treatment-related and gender-related differences in SCH 23390 binding.

The three-way ANOVA indicated significant interactions between haloperidol injection, preweaning treatment and gender in 9 of 12 regions analyzed ($P \leq 0.05$, Table 2, G). Only the core and shell of the nucleus accumbens at 1.2 and the shell at 1.6 did not show significant 3-way interactions. However, these regions did show significant haloperidol by preweaning treatment (E) or sex by preweaning treatment (F) interactions. Selected regions also showed significant main effects of preweaning treatment at $P \leq 0.05$ (right column of Table 2, C).

Examination of haloperidol effects within each treatment and gender indicated that in vehicle-treated males, haloperidol increased SCH 23390 binding in 1 region, the nucleus accumbens shell and core at 1.2 ($P \leq 0.01$, *t*-test). Haloperidol did not significantly alter SCH 23390 binding in cocaine-treated males while in GBR 12909-treated males, 6 of 12 regions showed decreases in SCH 23390 binding after haloperidol injection. Haloperidol did not significantly alter SCH 23390 binding in any female group ($P > 0.01$).

4. Discussion

Chronic haloperidol produced the expected increase in dopamine D_2 receptors primarily in terminal-rich regions such as the caudate putamen and nucleus accumbens (Burt et al., 1977; Chipkin et al., 1987; MacLennan et al., 1988; Angulo et al., 1991; Bernard et al., 1991; Rivest et al., 1995). Increases in dopamine D_2 receptor mRNA expression have been identified following haloperidol administration but the data are controversial (Angulo et al., 1991; Bernard et al., 1991; Fox et al., 1994; Damask et al., 1996). Others suggest that haloperidol upregulates dopamine D_2 receptor binding sites by an apparent delay in the breakdown of those receptors (Burt et al., 1977; Chipkin et al., 1987; MacLennan et al., 1988). While the mechanism by which haloperidol produces receptor upregulation is not known, increasing synthesis or decreasing breakdown of receptors may be involved. In males, cocaine increased the number of areas showing significant increases in dopamine D_2 receptor density indicating that the dopaminoceptive cells certainly are responsive. If haloperidol acts to retard breakdown of dopamine D_2 receptors as studies suggested (Angulo et al., 1991), the present data suggest that this effect is more widespread in cocaine-treated males. Not only were portions of the accumbens and inferomedial caudate affected by haloperidol, but also the entire caudate and substantia nigra pars reticulata showed increased raclopride binding in cocaine-treated males injected with haloperidol. GBR 12909 treatment in males produced an intermediate pattern of raclopride binding, which more closely resembled that seen in control males.

In females, haloperidol produced no statistically significant effects on raclopride binding while cocaine treatment enhanced the sensitivity to haloperidol in a single area, the rostral accumbens. GBR 12909 treatment in females, however, produced a widespread upregulation of the dopamine D_2 receptor throughout the caudate putamen and accumbens in response to haloperidol. The reduced effectiveness of haloperidol in females in general may relate to differential metabolism of the drug or to differences in the action of haloperidol in females compared to males. However, these arguments do not shed light on the dramatic response of the GBR 12909-treated females to haloperidol since it is unlikely, although possible, that the preweaning GBR 12909 treatment in females significantly altered haloperidol metabolism or its pharmacokinetics. Other postsynaptic mechanisms may be involved. While haloperidol administered to control females failed to alter raclopride binding, other studies have shown that females are more responsive to haloperidol than males. It is possible that dopamine D_2 receptor degradation rates are more rapid in females than males such that receptor levels returned to control levels within the 3 day wash out period (Di Paolo et al., 1981; Van Hartesveldt and Joyce, 1986; Morissette et al., 1990; Morissette and Di Paolo, 1993; Di Paolo, 1994; Rivest et al., 1995). While the pharmacological responses to both

Notes to Table 2:

The values are the means ± S.E.M. ($n = 7–8$/cell) in fmol/mg tissue.

Statistical comparisons were made by three-way analysis of variance (ANOVA), Dunnett tests and *t*-tests.

[a] Significant difference from vehicle-treated males, $P \leq 0.05$ by Dunnett.

[b] *p*-values of main effect of treatment in saline or haloperidol injected rats (2-way ANOVA). m: Significant interaction of sex by treatment.

[c] Significant difference between male and female in cocaine treatment groups. g: significant difference between male and female in GBR 12909 treatment groups ($P \leq 0.05$). *x* indicates significant differences by 3-way ANOVA $P \leq 0.05$. C: Main effect of preweaning treatment. E: interaction haloperidol by treatment. F: interaction sex by treatment. G: interaction haloperidol by sex by treatment.

[d] *P*-value (*t*-test) between saline and haloperidol groups which had same preweaning treatment.

[e] Indicates the distance from bregma based on atlas of Paxinos and Watson (1986).

haloperidol and cocaine are relatively similar in rats and humans, little information is available which would support or refute a gender difference in humans as we have observed for the rat. However, these data combined with other data we have collected in this model support the long-term effects of cocaine on function of the dopamine systems (Busidan and Dow-Edwards, in press; Dow-Edwards et al., 1993, 1996; Dow-Edwards and Hurd, 1998; Dow-Edwards and Busidan, in revision), an effect which should be of concern to those clinicians treating cocaine-exposed children.

Perhaps the most intriguing aspects of these results are the interactive effects of preweaning treatment and haloperidol injection on SCH 23390 binding which reflects dopamine D_1 receptor density. All but a restricted area of the nucleus accumbens showed significant 3 way interactions between preweaning treatment, gender and haloperidol administration. In control males, haloperidol significantly upregulated SCH 23390 binding in one region, the nucleus accumbens, core and shell at 1.2. Binding in all other brain regions was not affected by haloperidol. In the preweaning cocaine treatment group, haloperidol injection did not alter SCH 23390 binding in any region. Therefore, while the preweaning cocaine treatment increased responsiveness of the dopamine D_2 receptor system, the responsivity of the dopamine D_1 receptor system appeared to be dampened particularly in the nucleus accumbens. Other data have shown that the nucleus accumbens is unique in showing changes in dopamine D_1 receptor-mediated events in cocaine treated males. This dampening of dopamine D_1 receptor function may be related to the uncoupling of dopamine D_1 receptor from its Gs protein by developmental cocaine exposure (Wang et al., 1995). The treatment group primarily responsible for the 3 way interaction between treatment, gender and haloperidol is the GBR 12909-treated male group. Preweaning GBR 12909 treatment greatly increased basal (under saline condition) dopamine D_1 receptor binding densities in all regions examined in males (Table 2, a). The increase in basal dopamine D_1 receptor binding may indicate that preweaning GBR 12909 treatment may have initiated a proliferation of dopaminoceptive neurons. Examination of values for raclopride binding indicates that the density of dopamine D_2 receptors is 10–15% above that of the control in the saline-injected group, a difference which is not statistically significant. However, if GBR 12909 treatment did produce a proliferation of both pre and postsynaptic elements and if haloperidol increases dopamine release (as has been demonstrated using microdialysis (Imperato and Di Chiara, 1984, 1985; Imperato et al., 1993, 1994; Moghaddam and Bunny, 1993; Huang et al., 1997)), then the decrease in dopamine D_1 receptor density seen following haloperidol administration may be expected. Chronically high synaptic dopamine levels would be expected to produce a downregulation of the postsynaptic receptor. Thus, the decrease in SCH 23390 binding may indicate that, in the GBR 12909-treated males, haloperidol produced a dramatic increase in dopamine release which in turn produced a downregulation of dopamine D_1 receptors. Downregulation was not seen in dopamine D_2 receptors because they were occupied by haloperidol, the antagonist. Adult males which received similar GBR 12909 treatments during the preweaning period show reduced behavioral responses to dopamine D_1 receptor agonist challenge (Dow-Edwards and Busidan, in revision). However, GBR 12909-treated females and cocaine-treated males show similar alterations in locomotion following dopamine D_1 receptor agonist challenge and yet do not exhibit high basal densities of SCH 23390 binding (Dow-Edwards and Busidan, in revision). Thus, an inverse relationship between dopamine D_1 receptor density and behavioral response to dopamine D_1 agonist was not seen consistently. Finally, in females, none of the regions in any of the treatment groups showed statistically significant changes in dopamine D_1 receptor binding following haloperidol injection.

In summary, preweaning cocaine enhances haloperidol-induced upregulation of dopamine D_2 receptors in males only. Females, overall, are less sensitive to haloperidol than males. Preweaning GBR 12909, on the other hand, enhances the haloperidol-induced increase in dopamine D_2 receptor density in females and produces elevated levels of dopamine D_1 receptors which downregulate in response to chronic haloperidol injection. Overall changes in dorsal striatum are quite different from those in nucleus accumbens and selective inhibitors of the dopamine transporter like GBR 12909 are pharmacologically quite different from non-selective inhibitors such as cocaine. Inhibition of the dopamine transporter during preweaning period produces long-term alterations in the functional responsiveness of forebrain dopamine systems which are gender-specific.

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