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Individual Locomotor Response to Novelty Predicts Selective Alterations in D_1 and D_2 Receptors and mRNAs

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Rats that have a greater locomotor response to novelty (high responders, HR) have differences in measures of presynaptic dopamine transmission compared to low responders (LR) to a novel environment, including altered dopamine release and behavioral response to indirect dopamine agonists. This study examined the role of three dopamine terminal fields, the nucleus accumbens, striatum, and medial prefrontal cortex, in differences between HR and LR. In the first experiment, dopamine was infused directly into the nucleus accumbens (0, 3, 10, and 30 μg/side) or the striatum (0, 10, 30, and 100 μg/side). HR showed a greater behavioral response to both the 3 and 30 μg/side doses infused into the nucleus accumbens compared to LR. No differences between HR and LR were revealed by dopamine infusion into the striatum. In the second experiment, radioligand binding assays were performed to determine if differences exist between high and low responder rats in the B_{max} and/or K_D of radiolabeled antagonist ligands for the dopamine D_1 and/or D_2 receptors. There were fewer D_2 binding sites in the nucleus accumbens and fewer sites in the striatum in HR compared to LR. High responders showed a greater B_{max} for D_1 binding sites in the nucleus accumbens than LR. No differences in number of binding sites for D_1 receptors were observed between HR and LR in the striatum. No differences between HR and LR in D_2 or D_1 receptor binding were observed in the medial prefrontal cortex. There were no differences in K_D for any of the dopamine receptors in the regions examined. In experiment three, mRNA content for D_1 and D_2 receptors was evaluated in the nucleus accumbens, striatum, and the medial prefrontal cortex. HR showed a reduction in mRNA content for D_2 receptors compared to LR in the nucleus accumbens. No significant differences between HR and LR in D_2 mRNA content were observed in the striatum or medial frontal cortex. In addition, no differences between HR and LR were observed in D_1 mRNA content in the three regions examined. The final experiment evaluated the dopamine transporter. The B_{max} of binding to the dopamine transporter in the nucleus accumbens, striatum, or prefrontal cortex was not different between HR and LR. Likewise, the content for dopamine transporter mRNA in the substantia nigra did not differ. However, in the nucleus accumbens, but not the striatum, the velocity of dopamine uptake was greater in HR than in LR. It is concluded that alterations in postsynaptic dopamine transmission in the nucleus accumbens may partly mediate the locomotor response of an animal to a novel environment. The nucleus accumbens is of greater importance in differences between HR and LR than either the striatum or medial frontal cortex, and decreases in D_2 receptor density and mRNA are more marked than changes in D_1 receptors.

[Key words: individual differences, dopamine, receptor binding, mRNA, locomotor activity, striatum, nucleus accumbens, medial frontal cortex, D_1 receptors, D_2 receptors]

It has been shown that the vulnerability of rats to drugs of abuse such as amphetamine and cocaine can be predicted by their locomotor response to novelty. Thus, rats demonstrating a high locomotor response to novelty (high responders, HR) are more susceptible to the acquisition of amphetamine self-administration than subjects with a low locomotor response to novelty (low responders, LR) (Piazza et al., 1989). Recent experiments have shown variation in the dopaminergic system between HR and LR. Differences between HR and LR occur in both stimulated and basal dopamine levels in the nucleus accumbens, striatum, and medial prefrontal cortex (Bradberry et al., 1991; Hooks et al., 1991a, 1992a; Piazza et al., 1991). HR manifest a greater increase in extracellular dopamine levels to an amphetamine or cocaine challenge compared to LR, and HR have a greater basal level of dopamine in the nucleus accumbens than LR. In addition, amphetamine infusions into the nucleus accumbens produce greater behavioral activation in HR than LR (Hooks and Kalivas, 1994). Finally, Miserendino et al. (1993) find that the rate-limiting enzyme for dopamine synthesis, tyrosine hydroxylase, is elevated in the nucleus accumbens of HR.

The behavioral differences between HR and LR rats cannot be explained by variation in tissue and extracellular neurotransmitter concentrations alone. While some studies have shown a correlation between the locomotor response to novelty and the change in dopamine levels following pharmacological challenges, this only accounts for about 50–60% of the variation between subjects (Hooks et al., 1991a, 1992a; Piazza et al., 1991). In addition, changes in either extracellular or tissue basal levels of dopamine explain less than 60% of the variation (based on r^2) in locomotor response to novelty. Moreover, the extracellular

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concentration of dopamine following acute cocaine challenges does not correlate with the increase in locomotor activity (Kalivas and Duffy, 1990; Hooks et al., 1992a). These data indicate that variation in tissue and extracellular neurotransmitter levels alone cannot adequately account for all the variation in responsiveness to drugs of abuse between HR and LR rats.

In the present study, it was hypothesized that the response to released dopamine may differ between HR and LR and may contribute to the divergent behavioral response to novelty. Initial evaluation of this hypothesis was made by measuring the behavioral response to the microinjection of dopamine into the nucleus accumbens and striatum of HR and LR. Because this behavioral study revealed differences between HR and LR, the density and K_D of D_1 and D_2 dopamine antagonist binding sites were measured in tissue homogenates from the nucleus accumbens, striatum, and medial prefrontal cortex. The expression of mRNA encoding D_1 and D_2 receptors was measured in the three dopamine terminal fields using Northern blot analysis. Finally, involvement of the dopamine transporter was examined by measuring binding density, mRNA content, and the velocity of dopamine uptake.

Materials and Methods

Animal housing and surgery. Male Sprague–Dawley rats (Simonsen, Gilroy, CA, experiments 1, 3, and 4; Harlan–Sprague–Dawley, experiment 2) weighing 275–320 gm were used throughout the studies. Subjects were housed three or four per cage and maintained on a 12 hr light/dark cycle, and had free access to food and water.

Rats that were to be used for intracranial infusions were anesthetized with Equithesin and mounted in a stereotaxic apparatus (David Kopf, Torrance, CA). Chronic bilateral stainless steel cannulas (26 gauge) were implanted 1 mm above the nucleus accumbens, AP +3.4 from bregma, Lat ±3.1, Vert −6.5 from dura, with the incisor bar set at +5 mm (Pellegrino et al., 1979) or the striatum, AP +2.6 from bregma, Lat ±3.0, Vert −3.5 from dura with the incisor bar set at +5 mm (Pellegrino et al., 1979). The guide cannulas were secured in place with the use of skull screws and dental cement. Removable stylets (33 gauge) were placed in the guide cannulas. The wounds were sutured, and the rats were allowed a minimum of 1 week recovery prior to beginning experimentation.

Behavioral and microinjection procedures and histology. Subjects were screened for locomotor response to novelty in Plexiglas photocell cages between 0900 and 1300 hr in all four experiments. The photocell apparatus (Omnitech Electronics, Columbus, OH) consisted of 12 photocell cages operated simultaneously via Apple IIe computers. Each cage was equipped with 16 photocells that measured horizontal activity (eight front to back and eight side to side) located 2 cm off the cage floor and eight photocell beams that measured vertical activity (located in one direction) located 8 cm above the cage floor. Photocell cages were constructed of clear Plexiglas and measured 40 × 40 × 30 cm. The photocell beams located 2 cm above the floor were used to determine horizontal counts (number of times the beams were broken) and distance traveled (an estimate of locomotion determined by breaking adjacent photocell beams). Vertical counts were determined by the number of times the subject broke the beams located 8 cm above the floor. Subjects were placed in the photocell cages for a 1 hr period and were classified as HR if their locomotor response to novelty was in the upper 50% of the population screened. LR were subjects whose locomotor response to novelty was in the lower 50% screened (Hooks et al., 1991b).

Beginning 3 d following novelty screening, subjects that had been implanted with infusion cannulas received a series of dopamine infusions (0, 3, 10, and 30 μg/side for the nucleus accumbens or 0, 10, 30, and 100 μg/side for the striatum). Dopamine was dissolved in 0.9% sterile saline. After a 1 hr habituation period, rats received an infusion of saline or dopamine into either the nucleus accumbens or the striatum. Each rat received four separate trials that were randomized using a Latin-square design and separated by a minimum 72 hr intertrial interval. Infusions were via bilateral infusion cannulas (33 gauge stainless steel) inserted 1 mm below the guide cannulas. The infusions (Sage infusion pump) were made over 60 sec in a volume of 0.5 μl/side. Twenty seconds after discontinuing the infusion the rat was returned to the photocell cage for 2 hr and activity monitored. All subjects were tested with cocaine (15 mg/kg, i.p.) using a similar procedure 72 hr following the last intracerebral infusion. After this final behavioral trial, the rats were given an overdose of pentobarbital (100 mg/kg, i.p.) and their brains perfused via intracardiac infusion of 10% formalin. Coronal brain sections (100 μm thick) were made and stained with cresyl violet for identification of cannula tip location by an individual unaware of the rat's behavioral response. Although the atlas of Pellegrino et al. (1979) was used to obtain surgical coordinates, the atlas of Paxinos and Watson (1986) was used to plot the location of cannula times because it provided more precise definition of brain nuclei and subregions.

Radioligand binding assay procedure. Tissue was taken from the nucleus accumbens, striatum, and medial prefrontal cortex 7–10 d after screening for HR and LR. The tissue was dissected on a cooled metal plate and rapidly frozen at −70°C until use. Tissue was used within 7 d of collection. Pooled tissue samples were added to wash buffer (1:20 w/v) and a polytron was used to disrupt it. Samples were centrifuged at 50,000 × *g* at 4°C for 15 min. The pellet was washed by resuspension in wash buffer (50 mM Tris-HCl, 5 mM EDTA, and 50 mM NaCl at pH 7.5 for SCH 23390 and raclopride, and 50 mM Tris-HCl and 50 mM NaCl at pH 7.5 for GBR 12935). The final pellet was resuspended in assay buffer (50 mM Tris-HCl, 100 nM ketanserin, 120 mM NaCl, 5 mM KCl, 2 mM $MgCl_2$, 2 mM $CaCl_2$, and 0.5 mM EDTA adjusted to pH 7.8, for SCH 23390 and raclopride, and 50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, and 0.01% bovine serum albumin, adjusted to pH 7.8, for GBR 12935).

Binding was initiated by addition of tissue (150 μl) to assay buffer containing radiolabeled ligand with a total assay volume of 1 ml. For D_1 receptor binding, nine concentrations of ^{3}H-SCH 23390 (0.2–6.0 nM, 63 Ci/mmol; New England Nuclear) were incubated for 60 min at 37°C (George et al., 1991). Flupentixol (1 μM final concentration; Research Biochemicals Incorporated) was used as a competitive inhibitor to determine nonspecific binding. Nine concentrations of ^{3}H-raclopride (0.2–10 nM, 71.3 Ci/mmol; New England Nuclear) were incubated for 60 min at 25°C (Dewar et al., 1989; George et al., 1991). Butaclamol (1 μM final concentration; Research Biochemicals Incorporated) was used as a competitive inhibitor to determine nonspecific binding. Nine concentrations of ^{3}H-GBR 12935 (0.2–10 nM, 28.7 Ci/mmol; New England Nuclear) were incubated for 45 min at 25°C (Allard et al., 1991). GBR 12909 (5 μM final concentration; Research Biochemicals Incorporated) was used as a competitive inhibitor to determine nonspecific binding. Incubation was terminated by addition of 5 ml of chilled wash buffer and rapid filtration through Whatman GF/B filters using a Millipore manifold. Filters were presoaked in assay buffer. Each filtration was followed by two rinses with 5 ml of cold buffer. Filters were placed in scintillation vials and 6 ml of scintillation cocktail (ScintiVerse BioHP, Fisher, Fairlawn, NJ) was added. Vials were allowed to set for at least 6 hr before radioactivity was counted. Binding data were evaluated by the method of Scatchard (1949) using LUNDON software. For each ligand the tissue from 12–15 subjects (n = 12 for D_2 assays, n = 15 for D_1 assays, n = 15 for dopamine uptake site) was used. In the nucleus accumbens (HR = 5, LR = 4), striatum (HR = 6, LR = 6), and medial prefrontal cortex (HR = 6, LR = 6) four to six assays were conducted in duplicate for each ligand and region. Each assay used tissue pooled from two to four subjects. Protein content was measured by the method of Lowry et al. (1951).

RNA isolation and Northern blot analysis. Tissue was taken from the nucleus accumbens, striatum, medial prefrontal cortex, and substantia nigra 7–10 d following screening. The tissue was dissected on a cooled metal plate and rapidly frozen at −70°C until use. The concentration of mRNA was determined by the methods of Sorg et al. (1993). Briefly, total RNA was isolated (Bingham and Zachar, 1985). The concentration of total RNA was estimated by optical absorption (A_{260}); total content for the nucleus accumbens and striatum was between 3 and 5 μg from a single region and for the medial prefrontal cortex and substantia nigra 5–8 μg was used. Denatured total RNA was electrophoresed through a formaldehyde gel, transferred to a nylon membrane, and UV cross-linked. RNA was hybridized to ^{32}P-labeled oligonucleotide for D_1, D_2, or the dopamine transporter, and nonspecifically bound ^{32}P was washed from the membrane. The membranes were exposed to preflashed FUJI-RX film at −80°C with an intensifying screen using exposures that were within the linear range. To normalize data for loading unequal amounts of RNA in each lane of the gel, membranes were also hybridized to ^{32}P-labeled β-actin, whose mRNA levels were not altered as a result of

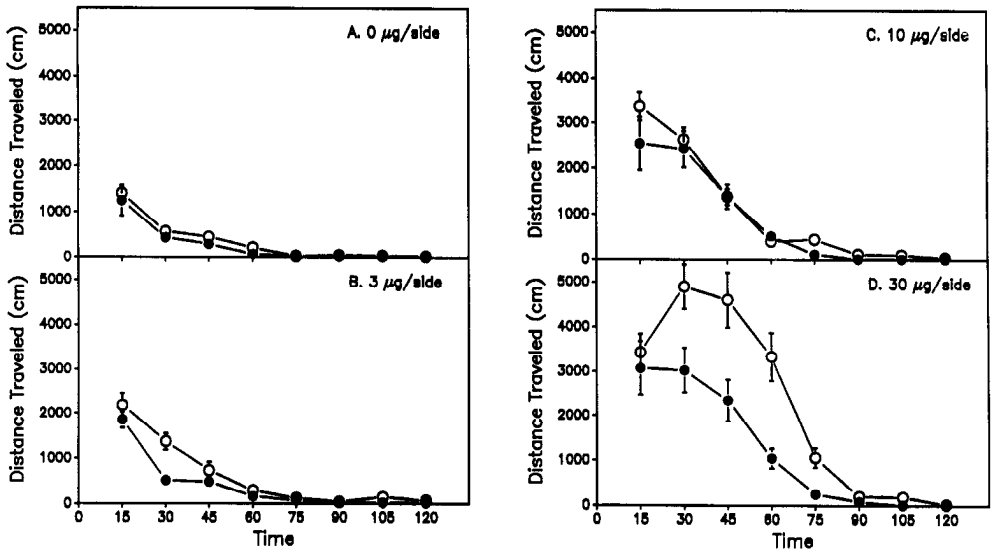

Figure 1. The effect of dopamine infusion (0, 3, 10, and 30 μg/side) into the nucleus accumbens on the time course of the increase in distance traveled (cm) elicited by dopamine. The data are shown as mean ± SEM. *Open symbols* refer to HR and *solid symbols* refer to LR.

response to novelty. Sizes of mRNA bands were determined using an RNA size standard (GIBCO–Bethesda Research Labs, Gaithersburg, MD). Relative optical density for each band was quantified by computerized videodensitometry. The system was calibrated using a photographic step tablet #2 (Eastman Kodak, Rochester, NY). For statistical analysis the measure of relative optical density was divided by the optical density of the β-actin and the resulting ratio expressed as a percentage of the LR rats.

Dopamine transporter velocity procedure. Tissue was taken from the nucleus accumbens and striatum 7–10 d after screening for HR and LR. The tissue from each hemisphere was analyzed separately. The transport of dopamine into nucleus accumbens and striatal suspensions was measured by rotating disk electrode voltammetry using previously described methods (McElvain and Schenk, 1992a,b; Meiergerd et al., 1993). Briefly, experiments were conducted by adding a pulse of 1.0 μM dopamine in 500 μl of tissue suspension in physiological buffer and monitoring its disappearance with a resolution of 100 msec. For the nucleus accumbens eight determinations were made for HR (tissue from five subjects) and five for LR (tissue from four subjects). For the dorsal striatum, six determinations were made for HR (tissue from four subjects) and six for LR (tissue from four subjects).

Statistical analysis. Total photocell counts were analyzed with a two-way analysis of variance (ANOVA) with repeated measures over dose. The time course was evaluated with a three-way ANOVA with repeated measures over time and dose. When significant ($p < 0.05$) F scores were revealed a simple main effects analysis was used for post hoc multiple comparisons (CRUNCH.4, Crunch Software Corp.). For analysis of receptor Scatchard plots, K_D and B_{max} values were calculated using linear regression. An unpaired two-tailed Student's t test was used for group comparison of K_D and B_{max} values obtained in receptor binding experiments. An unpaired two-tailed Student's t test was used to compare mRNA levels following Northern analysis. In addition, linear regression was performed to evaluate the relationship between mRNA levels and response to novelty. For dopamine uptake site kinetics, initial zero-order velocity of transport was obtained from the slope of the first 40 sec of the clearance profile (McElvain and Schenk, 1992a). Zero-order kinetics was defined as a linear plot of extracellular dopamine concentration versus time via linear regression analysis followed by an F test defining the significance of the regression analysis at the >95% confidence level and an intercept at $t = 0$ statistically indistinguishable from 1.0 μM.

Results

Dopamine infusions

Table 1 shows the behavioral differences between HR and LR in the response to novelty and a systemic cocaine challenge (15 mg/kg, i.p.) on three behavioral parameters: distance traveled (estimate of locomotion), horizontal activity (cumulative estimate of many horizontal behaviors such as locomotion, sniffing, and grooming), and vertical activity (estimate of rearing). The motor response to novelty was greater in HR with striatal cannulas in horizontal counts [$T(1, 13) = 4.28$, $p < 0.001$] and distance traveled [$T(1, 13) = 3.33$, $p < 0.01$], but not in vertical counts [$T(1, 13) = 0.001$, NS]. The HR with striatal cannulas did not show an overall greater number of horizontal counts in response to dopamine than LR as indicated by the lack of a main effect of novelty [$F(1, 13) = 0.006$, NS]. There was a novelty × dose interaction [$F(3, 45) = 4.74$, $p < 0.01$] due to a greater response of HR to saline infusion compared to LR. For distance traveled, a similar result was observed with no main effect of novelty [$F(1, 14) = 0.001$, NS], but a significant novelty × dose interaction [$F(1, 45) = 4.30$, $p < 0.01$]. The highest dose of dopamine (100 μg/side) microinjection into the striatum significantly increased all behaviors except vertical activity in HR. However, no significant difference between HR and LR was produced by any dose of dopamine except saline. For vertical activity there was neither a novelty [$F(1, 14) = 0.913$, NS] nor a novelty × dose interaction [$F(3, 45) = 1.29$, NS]. The response to a systemic cocaine challenge was significantly greater in all behavioral parameters in the HR versus LR [horizontal counts = $T(1, 13) = 3.26$, $p < 0.01$; distance traveled = $T(1, 13) = 3.48$, $p < 0.005$; vertical counts = $T(1, 13) = 3.15$, $p < 0.01$].

Table 1 also shows that dopamine microinjection produced a dose-dependent increase in all three behavioral estimates after injection into the nucleus accumbens of both HR and LR. The motor response to novelty was greater in HR with NACC implants in horizontal counts [$T(1, 17) = 5.32$, $p < 0.0001$], distance traveled [$T(1, 17) = 4.72$, $p < 0.005$], and vertical counts [$T(1, 17) = 4.47$, $p < 0.0005$]. The behavioral stimulation by dopamine in the nucleus accumbens was greater in horizontal and distance traveled in the HR compared to LR as indicated by a main effect of novelty [horizontal activities = $F(1, 18) = 7.29$, $p < 0.025$; distance traveled = $F(1, 18) = 8.88$, $p < 0.01$]. In addition, there was a novelty × dose interaction for all three behavioral measures [horizontal activity = $F(3, 57) = 3.32$, $p < 0.05$; distance traveled = $F(3, 57) = 5.23$, $p < 0.01$; vertical activity = $F(3, 57) = 2.88$, $p < 0.05$]. For distance traveled and horizontal activity a significant difference was measured after

Table 1. The effects of exposure to a novel environment, dopamine infusions, and cocaine (15 mg/kg, i.p.) on horizontal counts, distance traveled (cm), and vertical counts in HR and LR

	Horizontal counts		Distance traveled (cm)		Vertical counts	
	HR	LR	HR	LR	HR	LR
NACC						
Novelty	15844 ± 769*	10366 ± 606	9841 ± 539*	6450 ± 420	141 ± 8*	92 ± 7
Vehicle	6325 ± 698	5022 ± 716	2791 ± 302	2180 ± 437	33 ± 4	27 ± 5
3 μg/side	9076 ± 1137*	5747 ± 689	4978 ± 504*	3155 ± 369	46 ± 10	44 ± 15
10 μg/side	12502 ± 1761	10679 ± 1871	8515 ± 902	7050 ± 1175	81 ± 23	74 ± 23
30 μg/side	27187 ± 4089*	14925 ± 2397	17741 ± 2005*	9863 ± 1549	155 ± 28*	79 ± 15
Cocaine (i.p.)	44174 ± 3516*	24717 ± 3156	31096 ± 2390*	16898 ± 2662	191 ± 39*	72 ± 27
STR						
Novelty	14305 ± 556*	10224 ± 492	8339 ± 420*	6024 ± 362	100 ± 12	100 ± 7
Vehicle	8098 ± 1306*	4063 ± 442	3806 ± 385*	2263 ± 267	50 ± 13	18 ± 5
10 μg/side	5870 ± 572	5918 ± 365	3504 ± 469	3365 ± 215	41 ± 6	30 ± 4
30 μg/side	6583 ± 553	6513 ± 841	3338 ± 235	3038 ± 339	51 ± 9	48 ± 18
100 μg/side	12313 ± 1389	16632 ± 1283	5684 ± 787	7828 ± 694	61 ± 8	75 ± 6
Cocaine (i.p.)	49374 ± 4763*	29069 ± 1471	35513 ± 3490*	19715 ± 1595	357 ± 32*	266 ± 32

The number of subjects are, for NACC, HR = 9, LR = 10; STR, HR = 8, LR = 7. The numbers for novelty are the mean ± SEM counts for the initial 1 hr in the novel environment. The numbers for dopamine infusions and i.p. cocaine are the mean ± SEM for the 2 hr following drug treatment.

* Significantly greater number of counts ($p < 0.05$) exhibited by HR compared to LR.

3.0 and 30.0 μg/side of dopamine, while only the highest dose revealed a difference in vertical activity. The response to a cocaine challenge was significantly greater in all behavioral parameters in the HR versus LR [horizontal counts = $T(1, 17) = 3.58, p < 0.005$; distance traveled = $T(1, 17) = 3.47, p < 0.005$; vertical counts = $T(1, 13) = 2.18, p < 0.05$].

Figure 1 shows the time course of the effect of dopamine in the nucleus accumbens on distance traveled. The increase in distance traveled occurred within the first 60 min after injection. There were a main effect of novelty [$F(1, 18) = 8.876, p < 0.01$], novelty × dose interaction [$F(3, 57) = 4.87, p < 0.0005$], novelty × time interaction [$F(7, 589) = 2.841, p < 0.01$], and a novelty × dose × time three-way interaction [$F(21, 589) = 2.11, p < 0.005$].

Figure 2 illustrates the location of the cannula tips in the nucleus accumbens and striatum. In the nucleus accumbens the injectors were localized at and ventrolateral to the anterior commissure. Microinjections in the striatum were in the anterior-dorsal part of the striatum.

D_1 and D_2 receptor binding

Figure 3*A* shows that the B_{max} of ^{3}H-SCH23390 binding to D_1 receptors in the nucleus accumbens was 18% [$T(1, 7) = 2.96, p < 0.05$] higher in HR (780 ± 27 fmol/mg protein) compared to LR (660 ± 26). In contrast, the maximal level of binding to D_1 receptors did not differ between HR and LR rats in the striatum [$T(1, 10) = 0.90$, NS; HR = 1040 ± 105, LR = 1120 ± 98] or medial prefrontal cortex [$T(1, 10) = 0.02$, NS; HR = 51.2 ± 4.6, LR = 51.0 ± 4.9]. There was no significant difference in K_D between the two groups in the regions examined (Table 2). There was a difference between HR (16,002 ± 800) and LR (10,523 ± 572) in their locomotor response to novelty [$T(1, 22) = 5.63, p < 0.0001$].

The B_{max} for ^{3}H-raclopride binding to D_2 receptors in the nucleus accumbens was 51% less in HR rats (268 ± 26 fmol/mg protein) compared to LR rats (548 ± 49) [$T(1, 7) = 3.96, p < 0.05$; Fig. 3*B*]. This difference in B_{max} is revealed in the Scatchard plot depicted in Figure 4. In addition, the B_{max} for D_2 receptors in the striatum was 42% less in HR (412 ± 42) compared to LR (711 ± 62) [$T(1, 10) = 4.12, p < 0.01$; Fig. 3*B*]. No difference between maximal binding to D_2 receptors was observed between HR (30 ± 10) and LR (26 ± 8) in the medial prefrontal cortex [$T(1, 10) = 0.23$, NS]. However, high variation in the data may have obscured differences between HR and LR rats in the medial prefrontal cortex. There was no significant difference in K_D between the two groups in the brain regions examined (Table 2). There was a difference between HR (15,968 ± 763) and LR (10,112 ± 543) in their locomotor response to novelty [$T(1, 28) = 6.53, p < 0.0001$].

D_1 and D_2 mRNA analysis

There was a difference between HR (15,832 ± 809) and LR (10,111 ± 497) used for mRNA determination in their locomotor response to novelty [$T(1, 22) = 6.14, p < 0.0001$]. Figures 5 and 6 demonstrate the presence of a 40% reduction in the amount of mRNA for D_2 receptors in the nucleus accumbens in HR compared to LR [$T(1, 22) = 3.14, p < 0.05$]. Moreover, the level of D_2 mRNA was negatively correlated to the locomotor response to novelty ($r = -0.53, p < 0.05, N = 24$). Although HR showed 20% less mRNA than LR for the D_2

Table 2. The K_D values for the rat nucleus accumbens, striatum, and medial frontal cortex binding in tissue pools

	K_D (nM)		
Group	NACC	STR	MFC
D_1 (SCH 23390)			
HR	0.386 ± 0.027	0.274 ± 0.015	0.262 ± 0.021
LR	0.438 ± 0.021	0.298 ± 0.018	0.274 ± 0.031
D_2 (raclopride)			
HR	0.791 ± 0.092	0.574 ± 0.055	0.674 ± 0.082
LR	0.751 ± 0.081	0.657 ± 0.062	0.721 ± 0.077

There were no differences between HR and LR for any of the regions and ligands examined.

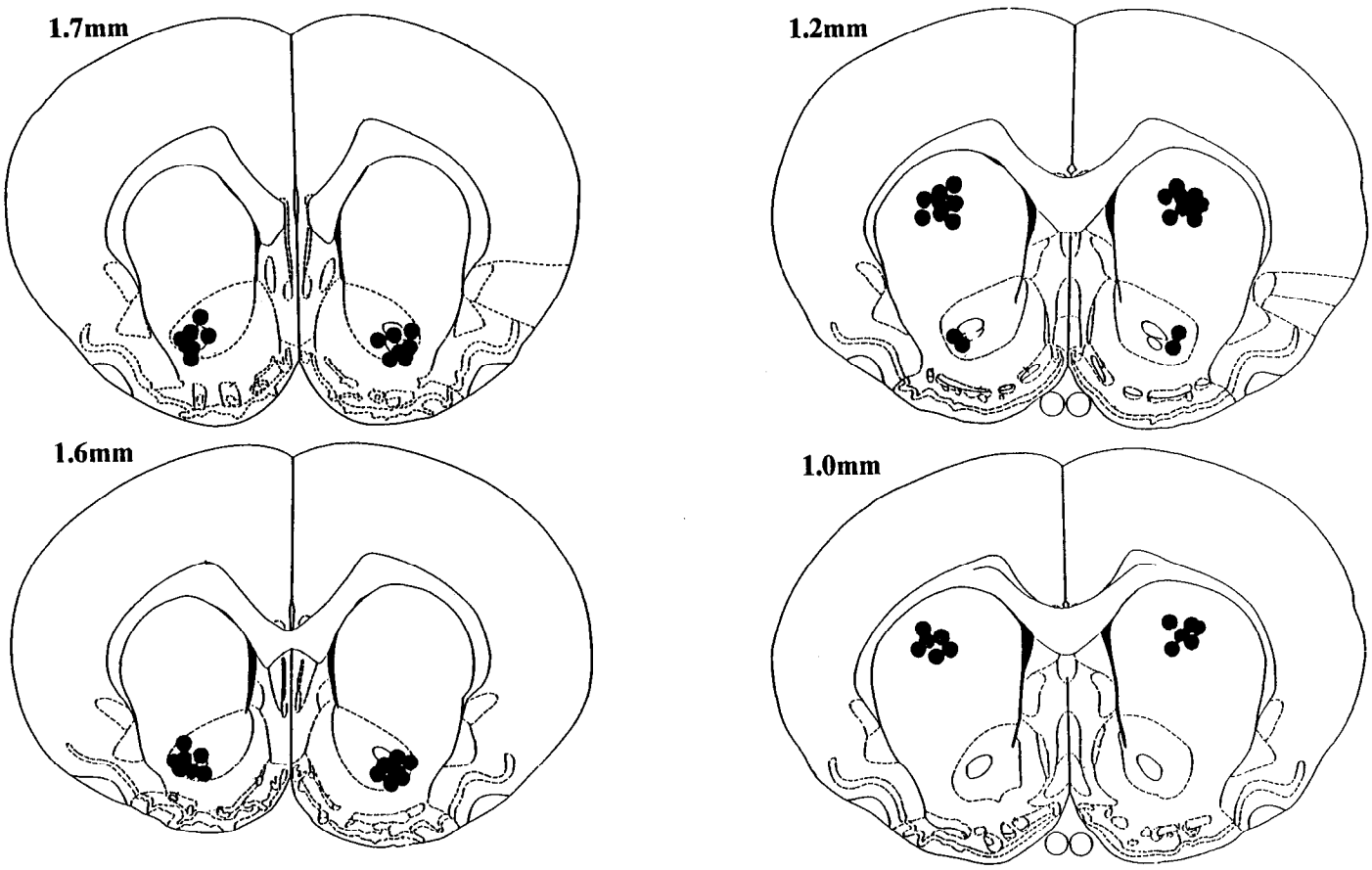

Figure 2. Location of microinjection cannulas in the nucleus accumbens and dorsal striatum. The drawings are based upon the atlas of Paxinos and Watson (1986). The distances anterior from bregma are indicated by each slice.

receptor in the striatum, this difference was not statistically significant [$T(1, 21) = 1.93, p < 0.07$]. In the medial prefrontal cortex there was no difference between the two groups in mRNA levels for the D_2 receptor [$T(1, 18) = 0.21$, NS]. No differences in absolute levels of the β-actin were observed between HR and LR in any of the three regions (data not shown, but see representative illustration in Fig. 6). Figure 6 shows representative Northern blots of D_2 and D_1 receptor mRNA in the nucleus accumbens, striatum, and medial frontal cortex and illustrates the reduction in D_2 mRNA present in HR in the nucleus accumbens.

There was a no difference for D_1 mRNA in HR compared to LR in the nucleus accumbens [$T(1, 20) = 1.21$, NS], medial prefrontal cortex [$T(1, 19) = 1.02$, NS], or striatum [$T(1, 20) = 0.57$, NS; Fig. 5]. No differences in absolute levels of β- actin were observed between HR and LR in any of the three regions (data not shown).

Dopamine transporter

The B_{max} for ^{3}H-GBR 12935 binding to the dopamine uptake site was not different between HR rats (952 ± 84 fmol/mg protein) and LR rats (882 ± 77) in the nucleus accumbens [$T(1, 7) = 0.96$, NS; Fig. 3*C*]. No differences between maximal binding to the dopamine uptake site were observed between HR (250 ± 88) and LR (248 ± 22) in the medial prefrontal cortex [$T(1, 10) = 0.13$, NS]. Although the B_{max} for the dopamine uptake site in the striatum was 20% less in HR (2301 ± 242) compared to LR (2771 ± 362), this difference did not reach statistical significance [$T(1, 7) = 1.96, p < 0.10$; Fig. 3*C*]. There was no significant difference in K_D between the two groups in the brain regions examined (Table 2). There was a significant difference between HR (15,753 ± 743) and LR (10,234 ± 569) in their locomotor response to novelty [$T(1, 28) = 7.21, p < 0.0001$].

There was a difference in locomotor response to novelty between the HR (15,789 ± 862) and LR (10,023 ± 542) used for dopamine transporter velocity determination [$T(1, 15) = 4.96, p < 0.0001$]. In tissue from the nucleus accumbens HR (177 ± 9 pmol/gm/sec) had a greater velocity of dopamine uptake than did LR [137 ± 12 pmol/gm/sec; $T(1, 11) = 3.85, p < 0.05$]. In the striatum, there was a nonsignificant trend for HR (429 ± 479 pmol/gm/sec) to have a greater dopamine uptake velocity than LR [307 ± 38 pmol/gm/sec; $T(1, 10) = 2.03, p < 0.10$]. Pooling HR and LR, the nucleus accumbens (160 ± 7 pmol/gm/sec) had lower dopamine uptake velocity than did the striatum (368 ± 29 pmol/gm/sec).

In the substantia nigra there was no difference between the two groups (HR, 105 ± 10, and LR, 100 ± 9) in mRNA levels for the dopamine uptake site [$T(1, 18) = 0.31$, NS]. No differences in absolute levels of β-actin were observed between HR and LR in the substantia nigra (data not shown).

Discussion

It has been previously reported that a number of presynaptic alterations in dopamine transmission are associated with rats demonstrating a high versus low locomotor response to a novel environment. There is an increase in the tissue concentrations

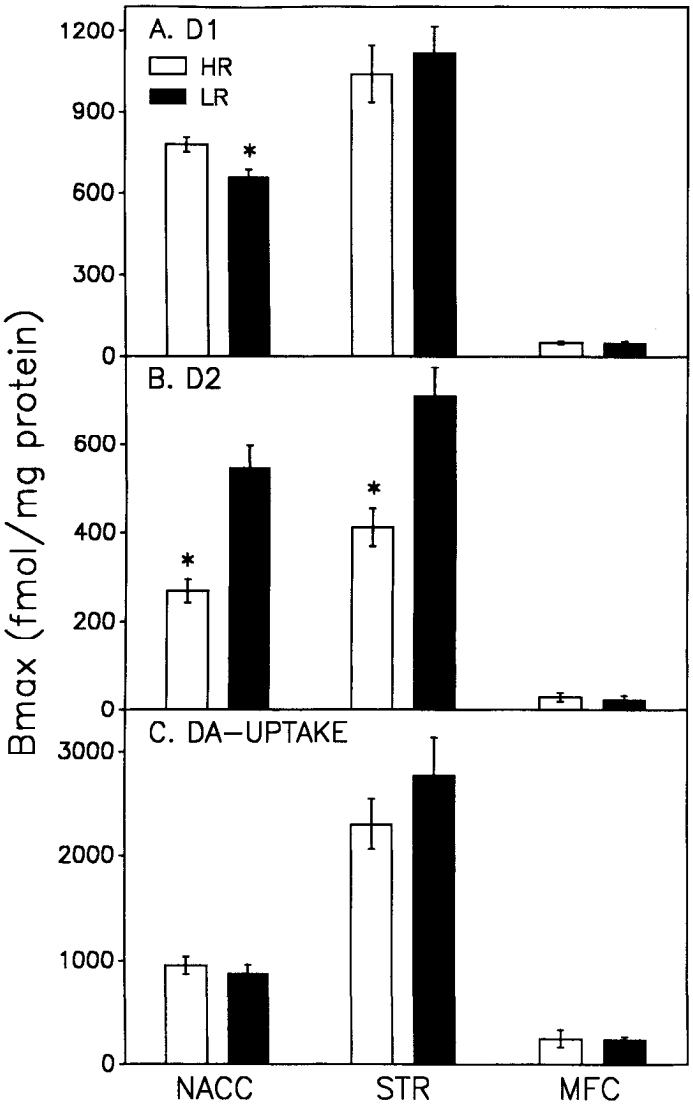

Figure 3. The B_{max} values for the rat nucleus accumbens, striatum, and medial frontal cortex binding in tissue pools using ^{3}H-SCH 23390 binding for the D_1 receptors (*A*), ^{3}H-raclopride binding for the D_2 receptors (*B*), and ^{3}H-GBR 12935 binding for the dopamine transporter (*C*). HR showed a greater B_{max} for D_1 receptors in the NACC. HR showed a smaller B_{max} for D_2 receptors in the NACC and STR. Values are expressed as mean ± SEM for four to six runs. *, significant difference between HR and LR ($p < 0.05$).

of tyrosine hydroxylase and dopamine, as well as an increase in extracellular dopamine content in the nucleus accumbens of HR compared to LR (Piazza et al., 1991; Hooks et al., 1992a; Miserendino et al., 1993). Also, the behavioral response to indirect dopamine agonists, such as amphetamine and cocaine, is augmented in HR versus LR (Piazza et al., 1989; Hooks et al., 1991a,b, 1992b). The present data show that in addition to changes in presynaptic transmission there are differences between HR and LR that are indicative of distinctions in postsynaptic elements of dopamine transmission. The augmented behavioral response to dopamine microinjection (3 and 30 μg/side) into the nucleus accumbens of HR indicates that the postsynaptic actions of dopamine are enhanced. Furthermore, differences in D_1 and D_2 dopamine receptors and mRNA in the nucleus accumbens pose a role for postsynaptic differences in accumbal dopamine transmission between HR and LR. The

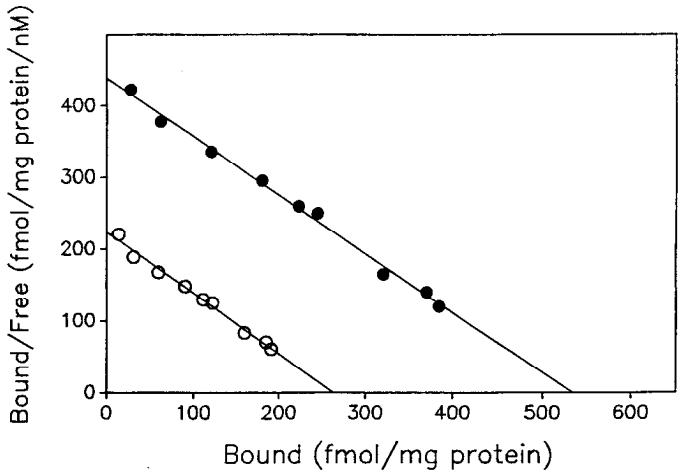

Figure 4. Typical Scatchard plots for HR (*solid circles*) and LR (*open circles*) following binding of NACC homogenates for the D_2 receptor. For HR in this plot, $B_{max} = 280$, $K_D = 0.804$. For LR in this plot, $B_{max} = 535$, $K_D = 0.793$.

increase in behavioral responsiveness to microinjected dopamine in HR is consistent with the previously reported elevation in measures of presynaptic dopamine transmission. Likewise, a slight increase in the B_{max} of ^{3}H-SCH 23390 binding to D_1 receptors in the nucleus accumbens is consistent with an augmentation in postsynaptic elements of dopamine transmission

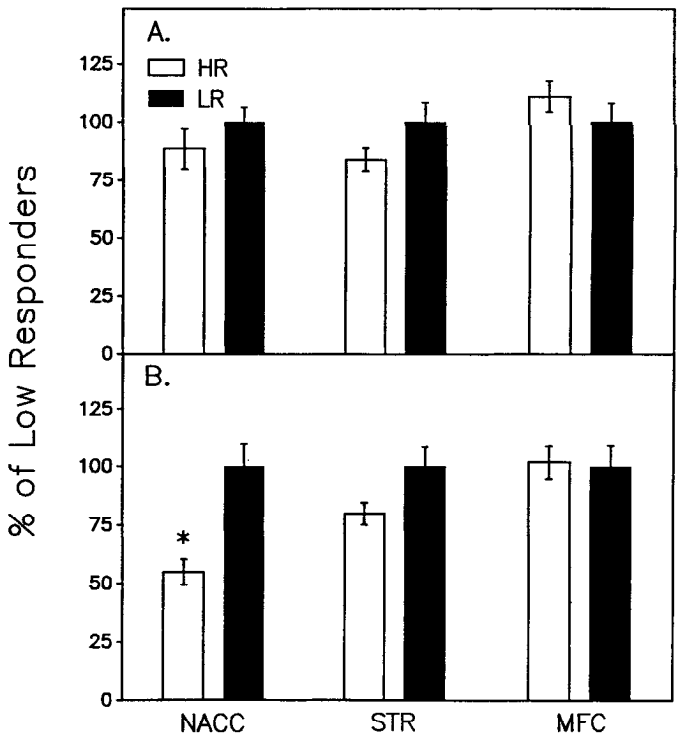

Figure 5. The normalized mRNA values for the rat nucleus accumbens, striatum, and medial frontal cortex using Northern analysis for the D_1 receptors (*A*) and the D_2 receptors (*B*). HR had a significantly less amount of mRNA for the D_2 receptors in the NACC than LR. This same trend was evident in the STR. No other differences between HR and LR in mRNA content were observed. Values are expressed as mean ± SEM of the ratio of D_1 or D_1 mRNA to β-actin normalized to the LR ratio ($n = 12$). *, significant difference between HR and LR ($p < 0.05$).

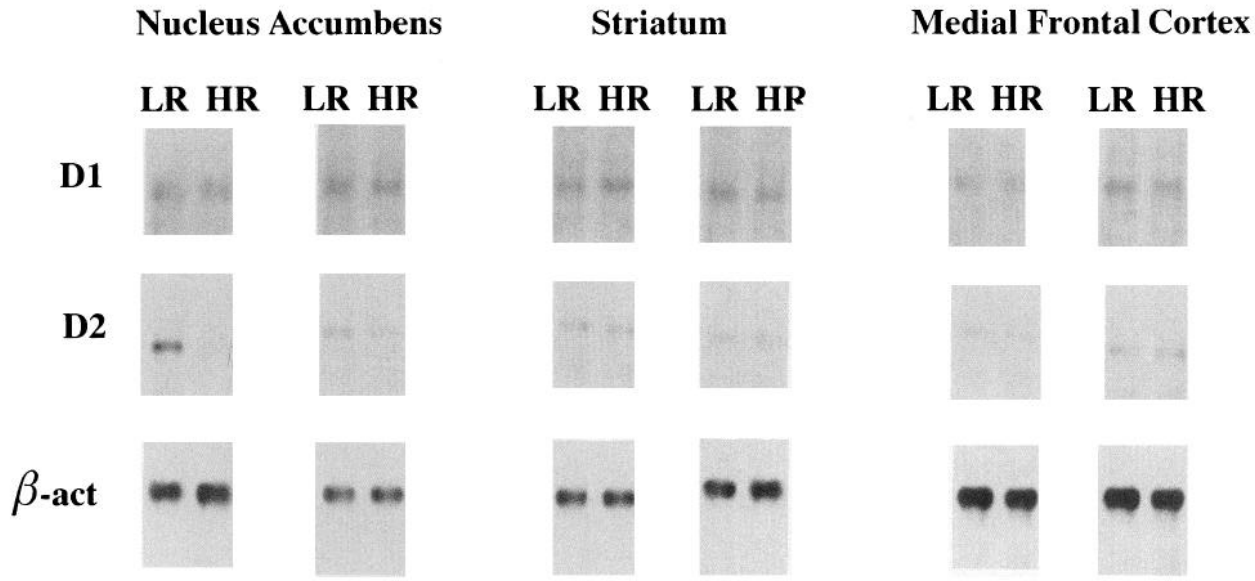

Figure 6. Typical Northern blots of dopamine D_1 receptors, dopamine D_2 receptors, and β-actin for the nucleus accumbens, striatum, and medial frontal cortex. There was approximately a 50% reduction in the intensity of the D_2 mRNA band for HR compared to the LR band in the nucleus accumbens. No differences in the band intensity for the D_1 receptor of the β-actin control probe were observed.

in HR. In contrast, the decrease in the B_{max} of ^{3}H-raclopride binding to D_2 receptors and in D_2 receptor mRNA measured in the nucleus accumbens of the HR is consistent with a diminution in pre- and/or postsynaptic dopamine transmission.

Considering the apparent increase in presynaptic dopamine transmission, which is indicated by the elevated basal extracellular levels of dopamine, the reduction in D_2 receptors and mRNA in HR can be explained as a downregulation in response to enhanced dopamine transmission. The continuous administration of dopamine agonists has been reported to reduce the B_{max} of antagonist binding to D_2 receptors in the nucleus accumbens and/or striatum (Ziegler et al., 1991). Thus, the increased level of extracellular dopamine content measured previously in the nucleus accumbens of HR is likely to produce a basal reduction in D_2 receptors. Furthermore, this homeostatic regulation is consistent with a decrease in the expression of mRNA for D_2 receptors.

Although the downregulation of D_2 receptors can be explained by an elevation in basal extracellular dopamine content, it is unclear why the motor stimulant response to dopamine is elevated in HR compared to LR in the presence of D_2 receptor downregulation. Four explanations can be considered to explain this apparent paradox. First, a significant portion of D_2 receptors expressed in the nucleus accumbens and striatum are located presynaptically on dopamine terminals (Roth, 1984; Dwoskin and Zahniser, 1986), and the reduction in ^{3}H-raclopride binding may result from a preferential decrease in presynaptic D_2 autoreceptors. Not only could a selective reduction in presynaptic receptors mask a relative increase in postsynaptic D_2 receptor density, but decreased inhibition of dopamine release by D_2 autoreceptors may account for the elevation in basal extracellular dopamine content in the nucleus accumbens of HR (Roth, 1984; Dwoskin and Zahniser, 1986; Kalivas and Duffy, 1991). While this explanation encompasses many observed differences in dopamine transmission between HR and LR, the facts that D_2 mRNA was drastically reduced in the nucleus accumbens of HR, and mRNA is located primarily in neuronal perikarya (Tobin and Khrestchatisky, 1989) argue that a significant portion of the reduction in D_2 receptor density arises from a decrease in postsynaptic receptors.

A second possibility is that, in spite of reduced receptor density, there may exist amplification in receptor transduction. While data do not exist directly evaluating this hypothesis, relevant analogies can be made with other models of increased susceptibility of drug reinforcement. Similar to HR versus LR (Piazza et al., 1989), Lewis rats demonstrate increased susceptibility to psychostimulant, opioid, or ethanol reinforcement compared to Fischer rats (George and Goldberg, 1989; Beitner-Johnson et al., 1992; Guitart et al., 1992). Likewise, rats treated chronically with cocaine or amphetamine demonstrate a greater susceptibility for psychostimulant self-administration (Horger et al., 1990, 1992; Piazza et al., 1990). Inasmuch as HR may resemble Lewis rats and rats pretreated chronically with psychostimulants (see Hooks and Kalivas, 1994, for discussion), it is interesting that both Lewis and psychostimulant-pretreated rats demonstrate an increase in accumbal tissue content of adenylate cyclase and cAMP-dependent protein kinase compared to controls (Beitner-Johnson et al., 1991, 1992; Terwilliger et al., 1991). Since D_1 receptor transduction is, in part, via enhanced activity of adenylate cyclase, an increase in this transduction mechanism could permit an augmented behavioral response to dopamine microinjection in HR in the presence of a reduction in D_2 receptors that are inhibitory on adenylate cyclase.

An additional explanation for the dissociation between dopamine-induced behavior and receptor density is that the spiny projection cells in the nucleus accumbens possessing a high density of D_2 receptors may be altered by input from other neurotransmitters such that the effect of D_1 or D_2 receptor stimulation is altered. A major afferent to these accumbal cells is exitatory amino acids arising from the prefrontal cortex, amygdala, and hippocampus, and at least the hippocampal and cortical afferents have been shown to impinge on the same accumbal cells as tyrosine hydroxylase–containing terminals (Pickel et al., 1988; Sesack and Pickel, 1990, 1992; Meredith et al., 1993).

In addition to dopamine, the motor activation following microinjection of the excitatory amino acid agonist AMPA into the nucleus accumbens is augmented in HR versus LR (Hooks and Kalivas, 1993). This poses the possibility that altered excitatory amino acid transmission may indirectly promote the postsynaptic actions of dopamine. Indeed, there exists electrophysiological evidence that dopamine and glutamate are functional postsynaptic antagonists (see Mogenson et al., 1993, for review), while behavioral studies primarily argue that the two transmitters are synergistic in the production of motor activity (Pulvirenti et al., 1989).

A final possibility is that the uptake of dopamine is reduced in HR such that even in the presence of reduced D_2 receptors an increase in extracellular dopamine in HR would be sufficient to produce a greater behavioral response. While we found no difference between HR and LR in dopamine transporter binding density in the nucleus accumbens or striatum or mRNA in the SN, the velocity of dopamine uptake was greater in HR than LR. Thus, inasmuch as dopamine uptake can influence the behavioral response to dopamine microinjections into the nucleus accumbens, the increase in dopamine uptake velocity should reduce the motor effect in HR.

Relative to the nucleus accumbens, the differences between HR and LR in dopamine receptors, mRNA, and behavioral response to dopamine in the striatum were less. Based upon the higher motor response of HR to a novel environment or psychostimulant challenge (Hooks et al., 1991a,b, 1992a) and the increased sensitivity to drug self-administration (Piazza et al., 1989), it is likely that the neural alterations distinguishing HR from LR would involve neural systems responsible for producing behavioral activation in response to motivationally relevant stimuli. By virtue of connectivity with both limbic and motor systems, the nucleus accumbens is frequently implicated in the translation of motivationally relevant behavior into adaptive motor responses (for reviews, see Kalivas et al., 1993; Koob et al., 1993; Mogenson et al., 1993). While the more extensive and selective anatomical involvement of the striatum with motor output may make it less likely to mediate the differences between HR and LR, the prefrontal cortex is thought to be intimately involved in modulating the expression of motivationally relevant behaviors (for reviews, see Deutch and Roth, 1990; Dworkin and Smith, 1992). Furthermore, alterations in cortical dopamine transmission modulate accumbal dopamine transmission (Pycock et al., 1980; Louilot et al., 1989; Rosin et al., 1992). Indeed, dopamine lesions of the prefrontal cortex predispose an animal to cocaine self-administration (Schenk et al., 1991) and HR rats have a reduction in dopamine metabolites in the prefrontal cortex compared to LR (Piazza et al., 1991). In spite of the intimate involvement of the prefrontal cortex in regulating dopamine transmission in the nucleus accumbens and accumbal-mediated behaviors, there was no difference between HR and LR detected in dopamine receptor density or mRNA in the prefrontal cortex.

In conclusion, the present data argue that differences in dopamine receptors exist between HR and LR. The changes were selective for the nucleus accumbens and involved primarily a reduction in D_2 receptor density and mRNA with relatively little alteration in D_1 receptors. In spite of the reduction in D_2 receptors, the behavioral response to dopamine microinjection into the nucleus accumbens was augmented in HR. This apparent mismatch between dopamine-dependent behavior and dopamine receptors poses the possible role of altered receptor transduction mechanisms or the involvement of other neurotransmitters in the nucleus accumbens known to modulate the postsynaptic effects of dopamine.

References

Beitner-Johnson D, Guitart X, Nestler EJ (1991) Dopaminergic brain reward regions of Lewis and Fischer rats display different levels of tyrosine hydroxylase and other morphine- and cocaine-regulated phosphoproteins. Brain Res 561:146–149.

Beitner-Johnson D, Guitart X, Nestler EJ (1992) Common intracellular actions of chronic morphine and cocaine in dopaminergic brain reward regions. Ann NY Acad Sci 654:70–87.

Bingham PM, Zachar Z (1985) Evidence that two mutations, W^{DZL} and ^{ZI}, affect synapsis-dependent genetic behavior of white and transcriptional regulatory mutations. Cell 40:819–823.

Bradberry CW, Gruen RJ, Berridge CW, Roth RH (1991) Individual differences in behavioral measures: correlation with nucleus accumbens dopamine measured by microdialysis. Pharmacol Biochem Behav 39:877–882.

Deutch AY, Roth RH (1990) The determinants of stress-induced activation of the prefrontal cortical dopamine system. Prog Brain Res 85:357–393.

Dewar KM, Montreuil B, Grondin L, Reader TA (1989) Dopamine D_2 receptors labeled with [^{3}H]raclopride in rat and rabbit brains. Equilibrium binding, kinetics, distribution and selectivity. J Pharmacol Exp Ther 250:696–706.

Dworkin SI, Smith JE (1992) Cortical regulation of self-administration. Ann NY Acad Sci 654:274–281.

Dwoskin LP, Zahniser NR (1986) Robust modulation of [^{3}H]dopamine release from rat striatal slices by D-2 dopamine receptors. J Pharmacol Exp Ther 239:442–453.

George FR, Goldberg SR (1989) Genetic approaches to the analysis of addiction processes. Trends Pharmacol Sci 40:571–580.

George FR, Porrino LJ, Ritz MC, Goldberg SR (1991) Inbred rat strains indicated different sites of action for cocaine and amphetamine locomotor stimulant effects. Psychopharmacology (Berlin) 104:457–462.

Guitart X, Beitner-Johnson D, Marby DW, Kosten TA, Nestler EJ (1992) Lewis and Fischer rat strains differ in basal levels of neurofilament proteins and their regulation by chronic morphine in the mesolimbic dopamine system. Synapse 12:242–253.

Hooks MS, Kalivas PW (1994) Involvement of dopamine and excitatory amino acid transmission in novelty-induced motor activity. J Pharmacol Exp Ther, in press.

Hooks MS, Jones GH, Smith AD, Neill DB, Justice JB Jr (1991a) Response to novelty predicts the locomotor and nucleus accumbens response to cocaine. Synapse 9:121–128.

Hooks MS, Jones GH, Smith AD, Neill DB, Justice JB (1991b) Individual differences in locomotor activity and sensitization. Pharmacol Biochem Behav 38:467–470.

Hooks MS, Colvin AC, Juncos JL, Justice JB Jr (1992a) Individual differences in basal and cocaine stimulated extracellular dopamine in the nucleus accumbens using quantitative microdialysis. Brain Res 587:306–312.

Hooks MS, Jones GH, Liem BJ, Justice JB Jr (1992b) Sensitization and individual differences to IP amphetamine, cocaine or caffeine following repeated intracranial amphetamine infusions. Pharmacol Biochem Behav 43:815–823.

Horger BA, Shelton K, Schenk S (1990) Preexposure sensitizes rats to the rewarding effects of cocaine. Pharmacol Biochem Behav 37: 707–711.

Horger BA, Giles MK, Schenk S (1992) Preexposure to amphetamine and nicotine predisposes rats to self-administer a low dose of cocaine. Psychopharmacology (Berlin) 107:271–276.

Kalivas PW, Duffy P (1990) The effect of acute and daily cocaine treatment on extracellular dopamine in the nucleus accumbens. Synapse 5:48–58.

Kalivas PW, Duffy P (1991) A comparison of axonal and somatodendritic dopamine release using *in vivo* dialysis. J Neurochem 56: 961–967.

Kalivas PW, Churchill L, Klitenick MA (1993) The circuitry mediating the translation of motivational stimuli into adaptive motor responses. In: Limbic motor circuits and neuropsychiatry (Kalivas PW, Barnes CD, eds), pp 237–288. Boca Raton, FL: CRC.

Koob GF, Robledo P, Markou A, Caine SB (1993) The mesocorticolimbic circuit in drug dependence and reward—a role for the extended amygdala? In: Limbic motor circuits and neuropsychiatry (Kalivas PW, Barnes CD, eds), pp 289–310. Boca Raton, FL: CRC.

Louilot A, le Moal M, Simon H (1989) Opposite influences of dopaminergic pathways to the prefrontal cortex or the septum on the dopaminergic transmission in the nucleus accumbens. An *in vivo* votammetric study. Neuroscience 29:45–56.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275.

McElvain JS, Schenk JO (1992a) A multisubstrate mechanism of striatal dopamine uptake and its inhibition by cocaine. Biochem Pharmacol 43:2189–2199.

McElvain JS, Schenk JO (1992b) Dopamine autoreceptor blockade by haloperidol and the apparent dynamics of endogenous dopamine release from and reuptake into striatal suspensions. Neuropharmacology 31:649–659.

Meiergerd SM, Patterson TA, Schenk JO (1993) D_2 receptors may modulate the function of the striatal transporter for dopamine: kinetic evidence from studies *in vitro* and *in vivo*. J Neurochem 61:764–767.

Meredith GE, Pennartz CMA, Groenewegen HJ (1993) The cellular framework for chemical signaling in the nucleus accumbens. Prog Brain Res, in press.

Miserendino MJD, Guitart X, Terwilliger RZ, Chi S, Nestler EJ (1994) Individual differences in locomotor activity are associated with levels of tyrosine hydroxylase and neurofilament proteins in the ventral tegmental area of Sprague–Dawley rats. Mol Cell Neurosci, in press.

Mogenson GJ, Brudzynski SM, Wu M, Yang CR, Yim CCY (1993) From motivation to action: a review of dopaminergic regulation of limbic-nucleus accumbens-pedunculopontine nucleus circuitries involved in limbic-motor integration. In: Limbic motor circuits and neuropsychiatry (Kalivas PW, Barnes CD, eds), pp 193–236. Boca Raton, FL: CRC.

Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates. New York: Academic.

Pellegrino LJ, Pellegrino AS, Cushman AJ (1979) A stereotaxic atlas of the rat brain. New York: Plenum.

Piazza PV, Deminiere JM, le Moal M, Simon H (1989) Factors that predict individual vulnerability to amphetamine self-administration. Science 29:1511–1513.

Piazza PV, Deminiere JM, le Moal M, Simon H (1990) Stress- and pharmacologically-induced behavioral sensitization increases vulnerability to acquisition of amphetamine self-administration. Brain Res 514:22–26.

Piazza PV, Rouge-Pont F, Deminiere PV, Kharoubi M, le Moal M, Simon S (1991) Dopaminergic activity is reduced in the prefrontal cortex and increased in the nucleus accumbens of rats predisposed to develop amphetamine self-administration. Brain Res 567:169–174.

Pickel VM, Towle AC, Joh TH, Chan J (1988) Gamma-aminobutyric acid in the medial nucleus accumbens: ultrastructural localization in neurons receiving monosynaptic input from catecholaminergic afferents. J Comp Neurol 272:1–14.

Pulvirenti L, Swerdlow NR, Koob GF (1989) Microinjections of a glutamate antagonist into the nucleus accumbens reduces psychostimulant locomotion in rats. Neurosci Lett 103:213–218.

Pycock CJ, Carter CJ, Kerwin RAW (1980) Effect of 6-hydroxydopamine lesions of the medial prefrontal cortex on neurotransmitter systems in subcortical sites in the rat. J Neurochem 34: 91–99.

Rosin DL, Clark WA, Goldstein M, Roth RH, Deutch AY (1992) Effects of 6-hydroxydopamine lesions of the prefrontal cortex on tyrosine hydroxylase activity in subcortical dopamine systems of the rat. Neuroscience 48:831–838.

Roth RH (1984) Dopamine autoreceptors: distribution, pharmacology and function. Ann NY Acad Sci 430:27–53.

Scatchard G (1949) The attractions of proteins for small molecules and ions. Ann NY Acad Sci 52:660–672.

Schenk S, Snow S, Horger BA (1991) Pre-exposure to amphetamine but not nicotine sensitizes rats to the motor activating effect of cocaine. Psychopharmacology (Berlin) 103:62–66.

Sesack SR, Pickel VM (1990) Synaptic input from opioid terminals to dopaminergic and nondopaminergic neurons in the ventral tegmental area of the rat. Eur J Pharmacol 183:208–209.

Sesack SR, Pickel VM (1992) Prefrontal cortical efferents in the rat synapse on unlabeled neuronal targets of catecholamine terminals in the nucleus accumbens seti and on dopamine neurons in the ventral tegmental area. J Comp Neurol 320:145–160.

Sorg BA, Chen S-Y, Kalivas PW (1993) Time course of tyrosine hydroxylase expression following behavioral sensitization to cocaine. J Pharmacol Exp Ther 266:424–430.

Terwilliger R, Beitner-Johnson D, Svarino KA, Crain SM, Nestler EJ (1991) A general role for adaptations in G-proteins and cyclic AMP system in mediating the chronic actions of morphine and cocaine on neuronal function. Brain Res 548:100–110.

Tobin AJ, Khrestchatisky M (1989) Gene expression in the mammalian nervous system. In: Basic neurochemistry (Segal G, Agranoff B, Albers RW, Molinoff P, eds), pp 417–428. New York: Raven.

Zeigler S, Lipton J, Toga A, Ellison G (1991) Continuous cocaine administration produces persisting changes in brain neurochemistry and behavior. Brain Res 555:27–35.