*Proc. Natl. Acad. Sci. USA*
Vol. 91, pp. 12564–12568, December 1994
Neurobiology

# Altered striatal function in a mutant mouse lacking $D_{1A}$ dopamine receptors

JOHN DRAGO*†, CHARLES R. GERFEN‡, JEAN E. LACHOWICZ§, HEINZ STEINER‡, TOM R. HOLLON§, PAUL E. LOVE*, GUCK T. OOI¶, ALEXANDER GRINBERG*, ERIC J. LEE*, SING PING HUANG*, PERRY F. BARTLETT||, PEDRO A. JOSE**, DAVID R. SIBLEY§, AND HEINER WESTPHAL*

*Laboratory of Mammalian Genes and Development, National Institute of Child Health and Human Development, ‡Laboratory of Cell Biology, National Institute of Mental Health, §Experimental Therapeutics Branch, National Institute of Neurological Disorders and Stroke, and ¶National Institute of Diabetes, Digestive and Kidney Disorders, National Institutes of Health, Bethesda, MD 20892; ||The Walter and Eliza Hall Institute of Medical Research, Parkville, Melbourne, Australia; and **Georgetown University Medical Center, Washington, DC 20007

*Communicated by Roscoe O. Brady, September 20, 1994*

**ABSTRACT** **Of the five known dopamine receptors, $D_{1A}$ and $D_2$ represent the major subtypes expressed in the striatum of the adult brain. Within the striatum, these two subtypes are differentially distributed in the two main neuronal populations that provide direct and indirect pathways between the striatum and the output nuclei of the basal ganglia. Movement disorders, including Parkinson disease and various dystonias, are thought to result from imbalanced activity in these pathways. Dopamine regulates movement through its differential effects on $D_{1A}$ receptors expressed by direct output neurons and $D_2$ receptors expressed by indirect output neurons. To further examine the interaction of $D_{1A}$ and $D_2$ neuronal pathways in the striatum, we used homologous recombination to generate mutant mice lacking functional $D_{1A}$ receptors ($D_{1A}-/-$). $D_{1A}-/-$ mutants are growth retarded and die shortly after weaning age unless their diet is supplemented with hydrated food. With such treatment the mice gain weight and survive to adulthood. Neurologically, $D_{1A}-/-$ mice exhibit normal coordination and locomotion, although they display a significant decrease in rearing behavior. Examination of the striatum revealed changes associated with the altered phenotype of these mutants. $D_{1A}$ receptor binding was absent in striatal sections from $D_{1A}-/-$ mice. Striatal neurons normally expressing functional $D_{1A}$ receptors are formed and persist in adult homozygous mutants. Moreover, substance P mRNA, which is colocalized specifically in striatal neurons with $D_{1A}$ receptors, is expressed at a reduced level. In contrast, levels of enkephalin mRNA, which is expressed in striatal neurons with $D_2$ receptors, are unaffected. These findings show that $D_{1A}-/-$ mice exhibit selective functional alterations in the striatal neurons giving rise to the direct striatal output pathway.**

The pivotal role played by dopamine receptors in the pathophysiology and treatment of Parkinson disease (1) and schizophrenia (2) and in the mode of action of addictive drugs such as amphetamine and cocaine (3, 4) is well established. Of the five known dopamine receptor subtypes (5), the $D_{1A}$ and $D_2$ receptors account for the vast majority of dopamine receptors (6) expressed in the striatum. The $D_{1A}$ (also known as $D_1$ in the primate system) and $D_2$ receptor subtypes are expressed mainly by spiny projection neurons, which account for 90–95% of the striatal neuron population (7). These striatal neurons may be subdivided into two major types on the basis of their axonal projections. One type provides a direct projection to the output nuclei of the basal ganglia: the substantia nigra and entopeduncular nucleus (the internal segment of the globus pallidus in primates). The other type provides projections to the globus pallidus (the external segment of the primate globus pallidus). As this latter type is connected indirectly to the output nuclei of the basal ganglia through connections with the subthalamic nucleus, the two output pathways are referred to as the direct and indirect output systems. Striatal neurons giving rise to the direct pathway express high levels of the $D_{1A}$ dopamine receptor subtype and the neuropeptides substance P and dynorphin, whereas neurons giving rise to the indirect pathway express high levels of the $D_2$ dopamine receptor and the peptide enkephalin (7). The levels of peptide expression in these neurons provide an assay of their activity (7), as neuropeptide levels correlated with firing rates in target neurons (1).

Current models suggest that imbalanced activity in the direct and indirect pathways is responsible for clinical movement disorders (8). A number of studies have demonstrated that dopamine oppositely effects these two output pathways through their differential expression of the $D_{1A}$ and $D_2$ receptors (7). For example, depletion of striatal dopamine with lesions of the nigrostriatal dopamine pathway in animal models of Parkinson disease results in reduced expression of substance P in direct output neurons and increased enkephalin expression in indirect striatal output neurons. Moreover, these changes may be selectively reversed with selective dopamine receptor agonist treatments, so that $D_1$ agonist treatment normalizes substance P levels whereas $D_2$ agonist treatment normalizes enkephalin levels (9). While these studies have demonstrated the differential role of $D_{1A}$ and $D_2$ receptors in striatal function, important questions concerning the interaction between these neuronal pathways remain. To provide an experimental animal model to address these questions, gene targeting by homologous recombination (10) was used to produce mutant mice which lack functional $D_{1A}$ receptors.

## MATERIALS AND METHODS

**Construction of Targeting Vector.** The targeting construct pK0.3 (Fig. 1) contains 7.0 kb of 129/Sv-derived $D_{1A}$ dopamine receptor genomic sequence in pPNT (11). The 3.4-kb 3′ homologous flank, consisting of an *Xba* I–*Bam*HI fragment isolated from clone pλA4.1, was subcloned between the *Xba* I and *Bam*HI sites of pPNT. The 5′ genomic flank was introduced into pPNT by using a number of intermediate subcloning steps, resulting in the subcloning of a 3.6-kb *Bam*HI–*Hin*dIII fragment (from pλ9 M) into the 5′ cloning site.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: ES cell, embryonic stem cell; ALS, acid-labile subunit of insulin-like growth factor/insulin-like growth factor-binding protein 3 ternary complex.

†To whom reprint requests should be addressed at: Laboratory of Mammalian Genes and Development, National Institute of Child Health and Human Development, Building 6B, Room 211, National Institutes of Health, Bethesda, MD 20892.

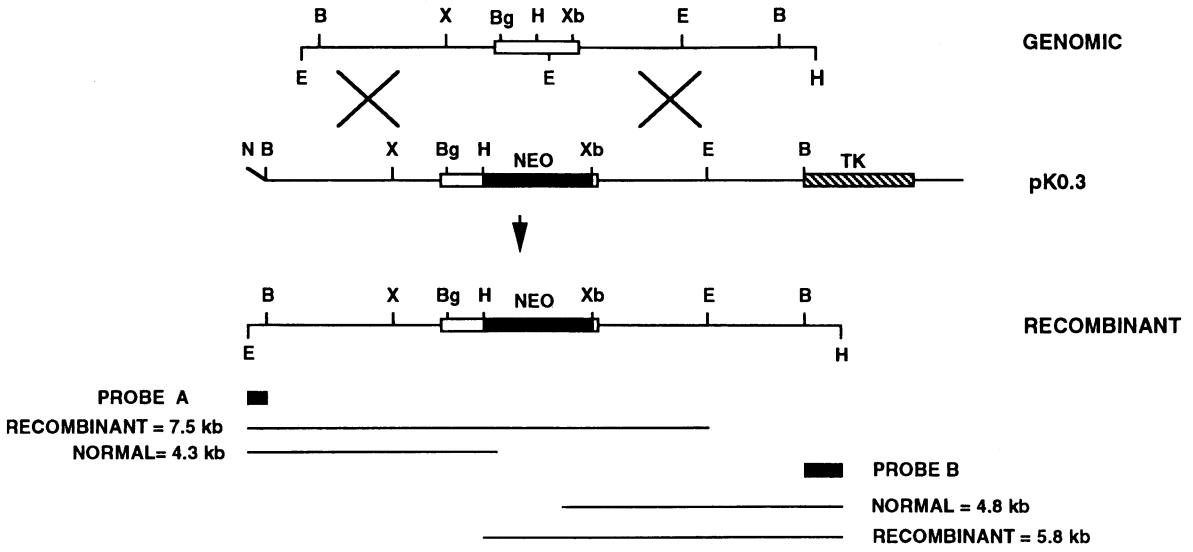


FIG. 1. Restriction map of the $D_{1A}$ gene, the homologous recombination targeting vector pK0.3, and the predicted restriction map following a successful recombination event. B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; X, *Xho* I; Xb, *Xba* I; N, *Not* I; NEO, neomycin phosphotransferase gene; TK, thymidine kinase gene. The probes used in Southern analysis and the predicted length of restriction fragments are also shown (see *Materials and Methods*).

**Gene Targeting and Generation of Mouse Mutants.** Tissue culture of embryonic stem (ES) cells and conditions for electroporation of the targeting construct have been described (12). By a strategy of simultaneous positive selection with G418 (350 $\mu$g/ml) and negative selection with ganciclovir (2 $\mu$M), the gene was successfully targeted in the J1 line (13) of ES cells (a gift from R. Jaenisch, Whitehead Institute). Homologous integration events were identified by Southern blotting (14). The hybridization probes and the predicted sizes of fragments generated by the endogenous and targeted alleles are shown in Fig. 1. Recombination was detected at the 5′ end by digesting genomic DNA with *Eco*RI and hybridizing the Southern blot with probe A (0.35-kb *Eco*RI–*Bam*HI fragment isolated from clone p$\lambda$9). The normal allele was 4.3 kb long and the recombinant allele was 7.5 kb. Correct recombination at the 3′ end was verified by digesting DNA with *Hin*dIII and hybridizing with probe B (0.6-kb *Bam*HI–*Hin*dIII fragment). The normal allele was 4.8 kb and the recombinant allele was 5.8 kb. The presence of a single integration event was demonstrated by probing a blot of *Hin*dIII-digested genomic DNA with a neomycin phosphotransferase gene-specific probe (probe NEO). Blastocyst injections were as described (13). Mice heterozygous for the $D_{1A}$ dopamine receptor deletion ($D_{1A}+/-$) were generated by mating male chimeras with C57BL/6 females. $D_{1A}-/-$ mutant mice were generated from heterozygous mouse intermatings.

**Neurological and Behavioral Analysis of Mutants.** Five- to six-week-old male mice were used for neurological and behavioral studies. All mice were provided with hydrated laboratory chow on the cage floor from the time of weaning and were caged individually for at least 2 days prior to testing. Neurological examination used a modification of a published procedure (15). Mice were examined for the presence of primitive reflexes. The righting reflex was tested in two ways: (*i*) the ability to land on four paws when dropped from an initial upside down position from a height of 40 cm onto a soft surface and (*ii*) an immediate turning onto four paws when released from a position in which mice were held with their back to the cage floor. Placing and grasp reflexes were tested as described (15). Coordination was tested by walking mice along a 0.6-cm-diameter wooden bar 15 cm above ground level and by using a tilting polystyrene platform. Normal mice placed on a platform will turn around and head up the platform as it is rotated from 0° to 90° over a 3-sec period. Locomotion was assessed by counting the number of line crossings in a square open field (60 cm × 60 cm, divided into nine squares) over a 15-min period. Grooming and rearing events were determined over the same time period in the open field. Akinesia was examined as described (16).

**Receptor Autoradiography and Homogenate Binding.** Binding of a radioiodinated $D_1$ receptor antagonist, $^{125}$I-labeled-SCH23982 (100 pM), on 10-$\mu$m coronal brain sections was assessed by autoradiography (17). Sections were exposed to reflection autoradiography film (DuPont/NEN) for 22 hr and the film was developed on a Kodak M35A X-Omat processor. Membrane assays were performed as described (18) with 0.04 mg of protein per ml and 1 $\mu$M (+)-butaclamol to define nonspecific binding.

***In Situ* Hybridization.** Oligonucleotide probes complementary to the mRNA encoding the $D_{1A}$ dopamine receptor (D1.1, 5′-CCTTCGGAGTCATCTTCCTCTCATACTGGA-AAGGGCAGGAGATAGCCC-3′, and D1.3, 5′-GACAG-GGTTTCCATTACCTGTGGTGGTCTGGCAGTTCTTG-GCATGGAC-3′) and the peptides substance P and enkephalin (19, 20) were labeled with $^{35}$S-tagged deoxyadenosine monophosphate (dAMP) tails of ≈25 bases in length (20) and used for *in situ* hybridization studies of striatal sections.

**Metabolic and Endocrine Analysis.** Blood urea nitrogen, a measure of renal function, and serum calcium, a reflection of parathyroid hormone activity, were determined by a DuPont Dimension autoanalyzer. Basal growth hormone activity was assessed by measuring liver mRNA levels for the acid-labile subunit of the 150-kDa insulin-like growth factor/insulin-like growth factor-binding protein 3 ternary complex (ALS) (21). Northern blots (22) were hybridized with a 1.3-kb rat ALS full-length cDNA probe.

## RESULTS

The $D_{1A}$ dopamine receptor-deficient mice were derived from ES cells in which one of the $D_{1A}$ receptor alleles was specifically mutated by homologous recombination. Disruption of the coding sequence was detected by screening independent ES cell clones for homologous integration of the targeting construct pKO.3. Positive clones contained an inactivated $D_{1A}$ receptor allele due to the insertion of the neomycin phosphotransferase gene into a *Hin*dIII restriction site located in the region of the $D_{1A}$ gene which encodes the

fifth predicted transmembrane domain (Fig. 1) and by the removal of 0.75 kb of downstream coding sequence. The excised genomic sequence encodes the predicted third intracytoplasmic loop, a region believed to interact with G proteins (5). Multiple clones contained a single neomycin phosphotransferase gene insert (Fig. 2*B*) flanked by the predicted recombinant 5′ (Fig. 2*A*) and 3′ (Fig. 2*B*) sequences. Two independently isolated ES cell clones, D39 and D134, were used to generate chimeric mice that transmitted the mutated allele to their offspring. Southern analysis of tail DNA from progeny of heterozygous matings revealed the predicted restriction pattern for the normal ($D_{1A}+/+$), $D_{1A}+/-$, and $D_{1A}-/-$ genotypes (Fig. 2*C*).

Although normal in weight up to 2 weeks of age, $D_{1A}-/-$ mice were significantly growth retarded at weaning age (week 3) (Fig. 3). $D_{1A}-/-$ mice appeared sick, with a poorly groomed coat and a hunched posture. Five out of six mice homozygous for the mutated allele caged under normal conditions, in which dry laboratory chow is supplied in food wells on the cage top, failed to gain weight and died within a week of weaning. $D_{1A}-/-$ mice ($n = 18$) separated from their $D_{1A}+/-$ or $D_{1A}+/+$ littermates and supplied with moistened laboratory chow looked healthy and gained weight, although they reached only 70% of the weight of their normal sex-matched littermate controls at 6 weeks (Fig. 3). There were no deaths in heterozygous or normal littermates over this same period of observation. The same phenotype was present in $D_{1A}-/-$ mice derived from two independently targeted ES cell clones. $D_{1A}-/-$ mice had normal righting, placing, and grasp reflexes. Coordination and the results of specific tests for akinesia were also normal. Although $D_{1A}-/-$ mutants appeared to be somewhat less active when examined in the open field test (15), the locomotor activity did not differ significantly from that of normal controls. However, $D_{1A}-/-$ mutants displayed significantly fewer rearing events than $D_{1A}+/+$ littermates [mean ± SEM, 19.0 ± 8.5 ($n = 11$) vs. 65.9 ± 9.3 ($n = 17$); $P < 0.002$, ANOVA]. Although reduced in number the rearing events appeared normal.

The $D_{1A}$ dopamine receptor is known to be expressed in the parathyroid gland (23, 24) and the proximal renal tubule (25). To determine whether altered parathyroid or renal function might be responsible for the growth-retarded phenotype, we measured serum calcium and blood urea nitrogen. The levels of calcium and blood urea nitrogen were not different among the groups. Furthermore, bone architecture and calcification were also normal, making hypoparathyroidism unlikely. Basal growth hormone activity, as reflected in liver ALS mRNA levels, was normal (data not shown).

Autoradiographic receptor binding with $^{125}$I-labeled SCH23982 demonstrated an absence of $D_1$ receptor binding

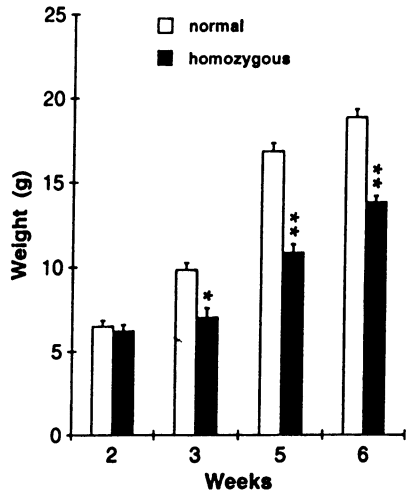


FIG. 3. Comparative postnatal weights of normal and $D_{1A}-/-$ mice. Weights (mean ± SEM) of normal ($n = 6$, open bars), and homozygous ($n = 5$, filled bars) males were recorded in the indicated postnatal weeks. The two groups differed in weight from week 3 onward. *, $P < 0.01$; **, $P < 0.001$ (two-factor ANOVA, *t* test).

in the brains of $D_{1A}-/-$ mice, whereas $D_{1A}+/+$ and $D_{1A}+/-$ animals displayed comparable signals in the caudate/putamen, nucleus accumbens, the olfactory tubercle (Fig. 4). Total binding in $D_{1A}-/-$ mutants was identical to that of nonspecific binding as defined by addition of 1 μM SCH23390 to the incubation buffer (data not shown). In a more sensitive and quantitative assay (homogenate receptor binding), heterozygous tissue displayed a reduced receptor number (Fig. 5), consistent with disruption of a single $D_{1A}$ dopamine receptor allele. The minor signal above background seen in $D_{1A}-/-$ mice probably reflects binding of [$^3$H]SCH23390 to 5-$HT_{2A}$ and 5-$HT_{2C}$ serotonin receptors, which exhibit significant affinity for this ligand (18) and are expressed at low levels in the striatum.

Brains were analyzed for expression of mRNA encoding the $D_{1A}$ receptor, the substance P, and enkephalin. Coronal sections through the striatum of normal mice showed $D_{1A}$ receptor mRNA distributed in the caudate/putamen, nucleus accumbens, and olfactory tubercle. Brain sections probed with an oligonucleotide probe (D1.3) complementary to mRNA normally transcribed from the gene sequence excluded from the targeting vector, and therefore absent from the disrupted allele, showed a reduced signal in $D_{1A}+/-$ and complete absence in $D_{1A}-/-$ animals (Fig. 4). However, striatal sections from $D_{1A}-/-$ mutants probed with an oli-

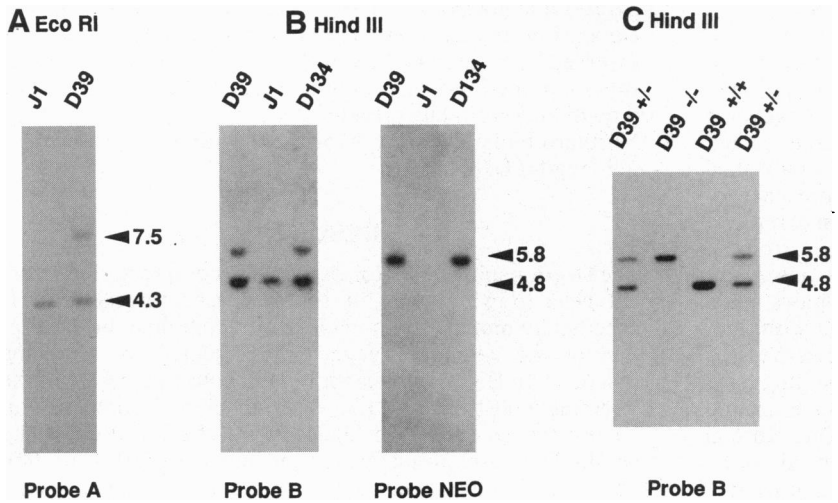


FIG. 2. Genomic analysis of targeted ES cell clones and mouse DNA. Recombination was detected at the 5′ end by digesting ES cell DNA with *Eco*RI and hybridizing the Southern blot with probe A (*A*). Recombination at the 3′ end was verified by digesting DNA with *Hin*dIII and hybridizing with probe B (*B Left*). A single integration event was confirmed by hybridizing *Hin*dIII-digested DNA with probe NEO (*B Right*). J1 represent wild-type-derived ES cell DNA; D39 and D134 represent two independently targeted ES cell clones. Genotyping of tail derived DNA revealed the expected pattern for normal D39 (+/+), heterozygous D39 (+/−), and homozygous D39 (−/−) animals (*C*). Numbers at right of each panel indicate fragment size in kilobases.

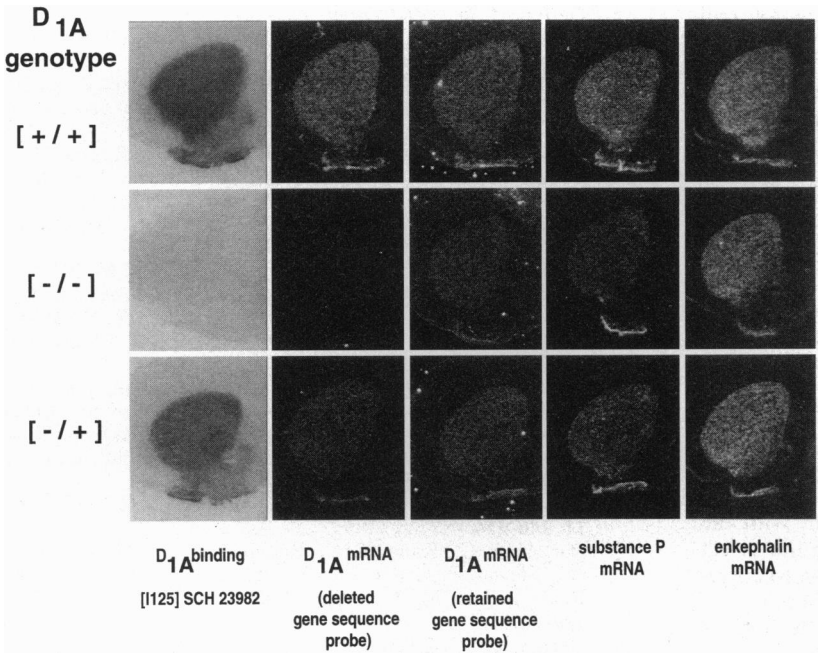


FIG. 4. Autoradiographic film labeling from coronal sections through the striatum from a normal mouse (+/+) (top row), a homozygous mouse (−/−) (middle row), and a heterozygous (+/−) $D_{1A}$ mouse (bottom row). Column 1, autoradiographic binding with $^{125}$I-labeled SCH23982; column 2, *in situ* hybridization histochemistry (IHSS) for $D_{1A}$ mRNA labeling obtained with a probe (D1.3) directed against the excised portion of the $D_{1A}$ gene; column 3, ISHH labeling of D1 mRNA obtained with a probe (D1.1) directed against the retained portion of the $D_{1A}$ gene; column 4, ISHH labeling of substance P mRNA; column 5, ISHH labeling of enkephalin mRNA.

gonucleotide (D1.1) complementary to mRNA transcribed from the upstream gene sequence retained in the 5′ homologous flank showed a definite although reduced hybridization signal (Fig. 4) relative to normal mice.

To assess the activity of the direct and indirect pathways, substance P and enkephalin neuropeptide mRNA levels were measured. The striatal expression of substance P was reduced in $D_{1A}-/-$ mutants, relative to $D_{1A}+/+$ mice (Fig. 4); the reduction was comparable in degree to the change seen in 6-hydroxydopamine-treated rats (9). In contrast, enkephalin mRNA (Fig. 4), encoding a neuropeptide essentially confined to the $D_2$ subpopulation of striatal neurons, was unaltered in homozygous mutants.

## DISCUSSION

The precise physiological role played by the $D_{1A}$ dopamine receptor is unclear, as studies performed to date have not specifically inactivated this receptor subtype. Lesion experiments in which the mesostriatal dopaminergic projections are destroyed by intracerebral injection of the neurotoxin 6-hydroxydopamine essentially deprive all dopamine receptor subtypes of endogenous ligand. In addition, drug studies with $D_1$ antagonists such as SCH23390 fail to discriminate between $D_{1A}$ and $D_{1B}$ subtypes (5). Homologous recombination was used to generate two independently derived mouse lines in which the $D_{1A}$ dopamine receptor gene was specifically inactivated. The lack of mRNA encoding the $D_{1A}$ receptor, together with the results of tissue autoradiography and striatal homogenate binding, confirm the lack of functional $D_{1A}$ receptors in $D_{1A}-/-$ mice.

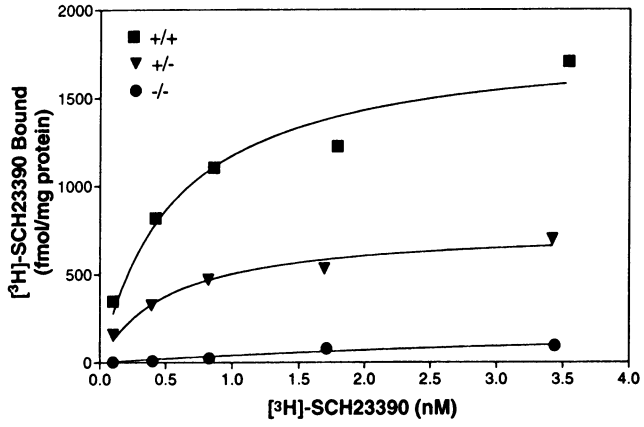


FIG. 5. Saturation binding analysis with [$^3$H]SCH23390, using striatal membranes prepared from $D_{1A}$ normal (+/+), heterozygous (+/−), and homozygous (−/−) mice. Specific binding is shown. Transformation of the data into Scatchard coordinates revealed the following binding parameters: normal, $K_d$ = 410 pM, $B_{max}$ = 1.7 pmol/mg of protein; heterozygous, $K_d$ = 380 pM, $B_{max}$ = 0.7 pmol/mg of protein. The specific binding in the homozygous animals was <10% of the total binding. These experiments were performed twice using two sets of animals, with similar results.

As demonstrated by the *in situ* hybridization studies with both the upstream $D_{1A}$ oligonucleotide and substance P probes, striatal neurons which normally express the $D_{1A}$ dopamine receptor gene are generated and persist in the adult striatum of homozygous mutants. This finding is significant given that a number of studies have suggested a role for dopamine in striatal development. For example, $D_{1A}$ receptors are expressed when striatal neurons are still neuroblastic in appearance (26). Also, $D_1$ receptor agonists inhibit growth cone motility (27) suggesting that modulation of dopamine receptor activity may perturb synaptogenesis and cell survival. Our results, however, suggest that $D_{1A}$ receptor integrity is not required for survival of striatal neurons.

The striking feature of $D_{1A}$ receptor null mutants is the growth retardation apparent at about 3 weeks of age. Homozygous mutants separated at weaning age and caged under normal laboratory conditions failed to gain weight and usually died within a week. In contrast, homozygous mutants caged away from heterozygous or normal littermates and given hydrated laboratory chow on the cage floor survived and gained weight. The growth retardation could not be explained on the basis of hypoparathyroidism, renal failure, or a reduction in growth hormone activity. Motivated behaviors, including feeding and drinking, have long been known to be critically linked to dopamine function. In fact, aphagia and adipsia are major consequences of dopamine depletion in animal models of Parkinson disease (28–30). Animals with severe dopamine depletion, as seen after bilateral administration of 6-hydroxydopamine, have to be fed via intragastric tube for a critical period after lesioning in order to survive (30, 31).

During the open-field test, $D_{1A}$ dopamine receptor null mutants showed a decreased number of rearing events. Rearing is considered part of a rodent's repertoire of spontaneous exploratory activities. Pharmacological studies have shown that the frequency of rearing events can be increased by $D_1$ receptor activation and decreased by $D_1$ receptor blockade (32–37). While some of these studies might indicate a somewhat higher sensitivity of rearing than locomotor activity for $D_1$ receptor blockade (32), it is clear that $D_1$ receptor modulation influences other behaviors including locomotion, sniffing, and grooming. Although there was substantial variation within the two groups, $D_{1A}-/-$ mice did not differ significantly from $D_{1A}+/+$ animals in the amount of locomotor activity shown in the open field. Our findings of decreased rearing rates and essentially unchanged locomotor activity in adult animals lacking functional $D_1$ receptors suggest that rearing may be more closely associated with $D_1$ receptor-mediated processes than locomotion.

Reduced substance P expression together with the finding of normal enkephalin mRNA levels is consistent with the postulated role of the $D_{1A}$ receptor in activating the direct pathway. The changes in peptide levels are known to correspond to changes in the metabolic activity in these pathways, as measured with 2-deoxyglucose metabolism (38, 39) and the induction of immediate early genes such as c-*fos* (40–42). Reduced substance P expression in $D_{1A}-/-$ mutants provides compelling *in vivo* evidence that the $D_{1A}$ receptor specifically modulates the activity of the direct striatonigral pathway. The specificity of this effect is further supported by the unaltered enkephalin mRNA levels expressed in $D_2$ receptor-regulated striatopallidal neurons.

$D_{1A}$ dopamine receptor mutant mice will most likely be useful for further study of basal ganglion function. For example, the lack of locomotor abnormalities may reflect some compensatory change in the $D_2$-modulated indirect pathway. Homozygous mutants may therefore provide a sensitive *in vivo* model system in which to screen novel psychotropic drugs for their potential to antagonize $D_2$ receptors and thereby produce movement disorders. Furthermore, indirect-acting dopamine receptor agonists such as cocaine and amphetamines are also thought to act via mechanisms mediated by $D_1$-like dopamine receptors (3, 43), although the exact molecular subtype is undefined. $D_{1A}-/-$ mice will therefore afford an opportunity to explore this question as well as examine $D_{1B}$ dopamine receptor function in a $D_{1A}$ dopamine receptor null background.

We thank Dr. Domenico Accili for helpful discussion, Dr. Douglas Feltner for critical review of the manuscript, and Errol Fields for technical help. The pPNT vector was a gift from Dr. Victor Tybulewicz. The J1 ES cells were a generous gift from Dr. Rudolf Jaenisch. J.D. is the recipient of a Basser Travelling Fellowship from the Royal Australasian College of Physicians.

1. Delong, M. R. & Wichmann, T. (1993) *Clin. Neurosci.* **1,** 18–26.
2. Seeman, P., Ulpian, C., Bergeron, C., Riederer, P., Jellinger, K., Gabriel, E., Reynolds, G. P. & Tourtellotte, W. W. (1984) *Science* **225,** 728–730.
3. Steiner, H. & Gerfen, C. R. (1993) *J. Neurosci.* **13,** 5066–5081.
4. Heikkila, R. E., Orlansky, H. & Cohen, G. (1975) *Biochem. Pharmacol.* **24,** 847–852.
5. Sibley, D. R. & Monsma, F. J., Jr. (1992) *Trends Pharmacol. Sci.* **13,** 61–69.
6. Levey, A. I., Hersch, S. M., Rye, D. B., Sunahara, R. K., Niznik, H. B., Kitt, C. A., Price, D. L., Maggio, R., Brann, M. R. & Ciliax, B. J. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 8861–8865.
7. Gerfen, C. R. (1992) *Trends NeuroSci.* **15,** 133–139.
8. Albin, R. L., Young, A. B. & Penney, J. B. (1989) *Trends NeuroSci.* **12,** 366–375.
9. Gerfen, C. R., Engber, T. M., Mahan, L. C., Susel, Z., Chase, T. N., Monsma, F. J., Jr., & Sibley, D. R. (1990) *Science* **250,** 1429–1432.
10. Thomas, K. R. & Capecchi, M. R. (1987) *Cell* **51,** 503–512.
11. Tybulewicz, V. L. J., Crawford, C. E., Jackson, P. K., Bronson, R. T. & Mulligan, R. C. (1991) *Cell* **65,** 1153–1163.
12. Love, P. E., Tremblay, M. L. & Westphal, H. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 9929–9933.
13. Li, E., Bestor, T. H. & Jaenisch, R. (1992) *Cell* **69,** 915–926.
14. Strauss, W. M. (1993) in *Current Protocols in Molecular Biology*, eds. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (Wiley, New York), pp. 2.2.1–2.2.2.
15. Huston, J. P. & Bures, J. (1983) in *Techniques and Basic Experiments for the Study of Brain and Behavior*, eds. Bures, J., Buresova, O. & Huston, J. (Elsevier, New York), pp. 77–86.
16. Meyer, M. E., Cottrell, G. A. & Van Hartesveldt, C. (1992) *Pharmacol. Biochem. Behav.* **41,** 507–510.
17. Scibilia, R. J., Lachowicz, J. E. & Kilts, C. D. (1992) *Synapse* **11,** 146–154.
18. Barton, A. C., Kang, H. C., Rinaudo, M. S., Monsma, F. J., Jr., Stewart-Fram, R. M., Macinko, J. A., Jr., Haugland, R. P., Ariano, M. A. & Sibley, D. R. (1991) *Brain Res.* **547,** 199–207.
19. Gerfen, C. R. & Young, W. S., III (1988) *Brain Res.* **460,** 161–167.
20. Young, W. S., III, Bonner, T. I. & Brann, M. R. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 9827–9831.
21. Chin, E., Zhou, J., Dai, J., Baxter, R. C. & Bondy, C. A. (1994) *Endocrinology* **134,** 2498–2504.
22. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162,** 156–159.
23. Kebabian, J. W. & Calne, D. B. (1979) *Nature (London)* **277,** 93–96.
24. Niznik, H. B. (1987) *Mol. Cell. Endocrinol.* **54,** 1–22.
25. Yamaguchi, I., Jose, P. A., Mouradian, M. M., Canessa, L. M., Monsma, F. J., Jr., Sibley, D. R., Takeyasu, K. & Felder, R. A. (1993) *Am. J. Physiol.* **264,** F280–F285.
26. Guennoun, R. & Bloch, B. (1992) *Mol. Brain Res.* **12,** 131–139.
27. Lankford, K. L., DeMello, F. G. & Klein, W. L. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 4567–4571.
28. Ungerstedt, U. (1971) *Acta Physiol. Scand. Suppl.* **367,** 95–122.
29. Fibiger, H. C., Zis, A. P. & McGeer, E. G. (1973) *Brain Res.* **55,** 135–148.
30. Marshall, J. F. & Teitelbaum, P. (1973) *Brain Res.* **55,** 229–233.
31. Zigmond, M. J., Stricker, E. M. & Berger, T. W. (1987) in *Animal Models of Dementia: A Synaptic Neurochemical Perspective*, ed. Coyle, J. T. (Liss, New York), pp. 1–38.
32. Hoffman, D. C. & Beninger, R. J. (1985) *Pharmacol. Biochem. Behav.* **22,** 341–342.
33. Breese, G. R., Duncan, G. E., Napier, T. C., Bondy, S. C., Iorio, L. C. & Mueller, R. A. (1987) *J. Pharmacol. Exp. Ther.* **240,** 167–176.
34. Dreher, J. K. & Jackson, D. M. (1989) *Brain Res.* **487,** 267–277.
35. Chandler, C. J., Wohab, W., Starr, B. S. & Starr, M. S. (1990) *Neuroscience* **38,** 437–445.
36. Meyer, M. E., Van Hartesveldt, C. & Potter, T. J. (1993) *Synapse* **13,** 310–314.
37. Meyer, M. E., Cottrell, G. A., Van Hartesveldt, C. & Potter, T. J. (1993) *Pharmacol. Biochem. Behav.* **44,** 429–432.
38. Kozlowski, M. R. & Marshall, J. F. (1980) *Brain Res.* **197,** 167–183.
39. Wooten, G. F. & Collins, R. C. (1983) *Brain Res.* **263,** 267–275.
40. Robertson, G. S., Vincent, S. R. & Fibiger, H. C. (1990) *Brain Res.* **523,** 288–290.
41. Robertson, G. S., Vincent, S. R. & Fibiger, H. C. (1992) *Neuroscience* **49,** 285–296.
42. Dragunow, M. & Faull, R. (1989) *J. Neurosci. Methods* **29,** 261–265.
43. Graybiel, A. M., Moratalla, R. & Robertson, H. A. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 6912–6916.