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Expression of the Weaver Gene in Dopamine-Containing Neural Systems is Dose-Dependent and Affects Both Striatal and Nonstriatal Regions

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In an earlier report we presented evidence pointing to a differential effect of the mutant gene *weaver* on the dopamine-containing fiber systems innervating the striatum. In mice homozygous for the weaver mutation, there is a severe loss of dopamine in the caudoputamen, the main target of the nigrostriatal system. By contrast, dopamine is entirely conserved in the nucleus accumbens, a target of the mesolimbic system, and is moderately affected in the olfactory tubercle. The present study shows that these defects in dopamine are gene dose-dependent, that they are established by the end of the first month of life, and that the losses are permanent and not progressive. As in homozygous weavers, the greatest defects in striatal dopamine in heterozygous weavers occur in the dorsolateral caudoputamen and the lateral olfactory tubercle.

The abnormalities in the striatal dopamine content of weaver mice are not accompanied by abnormalities in the turnover of dopamine, judging from measurements of the dopamine metabolite dihydroxyphenylacetic acid. Norepinephrine content is also normal in each striatal region. No deficits in striatal dopamine occur in mice homozygous for the mutant genes *staggerer* and *Purkinje cell degeneration*, which, like the weaver mutation, result in ataxia and cerebellar pathology.

A survey of nonstriatal regions in the weaver mice showed that the effects of the weaver gene on the dopamine-containing innervation of the forebrain are not confined to striatal targets but also extend to the septum and the hypothalamus. By contrast, dopamine in the frontal cortex, the amygdala, the olfactory bulb, and the retina is entirely spared. The pattern and extent of loss of dopamine in the weaver forebrain is thus region- and system-specific. In confirmation of our initial findings, a ca. 30% depletion of dopamine occurs in the weaver midbrain, the region containing the cell bodies of origin of the mesostriatal dopamine systems. A comparison of histofluorescent sections through weaver and control midbrains revealed a reduction of catecholamine-containing neurons in the pars compacta of the weaver animals.

These results point to a subpopulation of dopamine-containing neurons as primary targets of the weaver gene or as being closely associated with such primary targets. As a gene-dose effect has also been shown for the cerebellar granule cell loss in the weaver, the mutant gene must have at least 2 cellular targets. We suggest that the cerebellar and mesostriatal pathologies may be linked by a common molecular mechanism.

Two defects, one obvious and the other well hidden, are known to be present in the brains of mice that carry the autosomal recessive gene weaver. This mutation results in an atrophy of the cerebellum that is so severe in homozygous weavers that it can be detected after a glance at the intact brain. As was first reported by Sidman et al. in 1965, this macroscopic defect reflects an early postnatal loss of granule cells in the cerebellar cortex. A second abnormality in the weaver brain affects the forebrain and is concealed from view because it does not result in a marked atrophy or disruption of cellular architecture in the affected regions. This defect was first revealed biochemically as a selective reduction in the content of the neurotransmitter dopamine in brain (Lane et al., 1977), and was subsequently shown both by biochemical analysis and by fluorescence histochemistry to affect certain regions in the forebrain innervated by dopamine-containing fibers from the midbrain (Roffler-Tarlov and Graybiel, 1984; Schmidt et al., 1982).

In an earlier report, we presented evidence for a striking regional specificity in the effect of the weaver mutation on the content of dopamine in different parts of the striatum (Roffler-Tarlov and Graybiel, 1984). In the caudoputamen of homozygous mutants, there was an almost 70% reduction of dopamine relative to that in heterozygous controls, whereas in the nucleus accumbens, dopamine was entirely conserved. This anatomical pattern of dopamine loss strongly suggested that the weaver gene affects the dopamine-containing innervation of the forebrain according to recognized functional subdivisions (Ungerstedt, 1971).

We had 2 goals in the study reported here. First, we sought to determine whether the dopamine-containing nigrostriatal system affected in the weaver mice is likely to be a primary target of the weaver gene, as is true for the granule cells of the cerebellum. The cerebellar defect characteristic of homozygous weavers is manifested morphologically and biochemically in a less severe form in the heterozygous mice (Hatten et al., 1984a; Rakic and Sidman, 1973b, c; Rezai and Yoon, 1972; Roffler-Tarlov and Turey, 1982; Willinger and Margolis, 1985a, b). Thus, in the cerebellum, the presence of a single weaver allele results in a partial defect. To determine whether a heterozygote effect also occurs for the dopamine-containing innervation of the striatum, we measured the dopamine content of the caudoputamen, the nucleus accumbens, and the olfactory tubercle in each of the 3 genetic types, wv/wv, +/wv, and +/+. We also examined the dopamine content of these striatal districts in 2 other mutant mouse types exhibiting movement disorders as a result of autosomal recessive mutations expressed in the cerebellum. One of these, the staggerer mutant (sg), has in common with weaver a granule cell-deficient cerebellum (Sidman et al., 1962). The other, Purkinje cell degeneration (pcd), has in common an ataxia, but loses the Purkinje cells of the cerebellum

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(Mullen et al., 1976). By comparing these mutants to weavers, we tested for the possibilities that the dopamine defect in the weaver striatum might be attributable to abnormalities in motor behavior or cerebellar morphology, rather than being the result of a direct action of the weaver gene on dopamine-containing neurons or on cells affecting them.

Our second objective was to determine the range of action of the weaver gene on the dopamine-containing systems of the brain. We analyzed the content of dopamine present (1) in non-striatal as well as in striatal regions of the forebrain normally innervated by dopamine-containing neurons of the midbrain, and (2) in regions that have intrinsic dopamine-containing neurons distinct from the dopaminergic cell groups of the midbrain. Simultaneously, we measured the concentrations of dihydroxyphenylacetic acid (DOPAC) and norepinephrine to identify the nature and specificity of any abnormality in dopamine content found. To establish unequivocally whether the defect in striatal dopamine was accompanied by a loss of dopamine in the midbrain, we measured the dopamine content in samples of the midbrain containing neurons of cell groups A8, A9, and A10 of Dahlstrom and Fuxe (1964). The observations described below have been summarized, in part, in abstract form (Roffler-Tarlov and Graybiel, 1985b).

Materials and Methods

Mutant and control mice

Weaver, gene symbol wv

Homozygous weaver (wv/wv), heterozygous weaver (+/wv), and homozygous normal (+/+) animals were on a C57BL/6JLe-A^{wJ} × CBA/CaGnLeF_1 hybrid background. Mutant animals and most controls were obtained by breeding heterozygote pairs in a colony established with animals purchased from the Jackson Laboratory, Bar Harbor, Maine. Some +/+ animals were obtained from litters born of known +/+ C57BL6/CBA pairs. Mature wv/wv animals were identified behaviorally (Sidman, 1968; Sidman et al., 1965). The genetic type of the +/wv and +/+ animals was established postmortem by the macroscopic appearance and wet weight of the cerebellum. Compared to the cerebellum of the +/+ mouse, that of the +/wv appears misshapen and slightly deflated, particularly at the midline (Rakic and Sidman, 1973a, b; Rezai and Yoon, 1972) and is reduced in weight (Roffler-Tarlov and Turey, 1982).

Purkinje cell degeneration (pcd)

The Purkinje cell degeneration mutation, an autosomal recessive, was maintained on a C57BL6 background. Mutants and controls were obtained by breeding heterozygote pairs purchased from the Jackson Laboratory. The pcd/pcd animals were identified by their moderately ataxic gait, which appears on about postnatal day 25. The heterozygous pcd and homozygous normal animals were indistinguishable behaviorally and morphologically. As controls, we chose littermates with apparently normal motor behavior, which could have been +/+ or +/pcd.

Staggerer (sg)

The sg/sg mutants and controls were on C57BL6 stock and were kindly donated by Dr. Karl Herrup of Yale University. This autosomal recessive mutation results in a lack of granule cells (Sidman et al., 1962), reduced numbers of Purkinje cells (Herrup and Mullen, 1979), and malformation of the residual Purkinje cells (Landis and Sidman, 1978; Sotelo, 1975). The sg/sg animals were identified behaviorally by their marked ataxia. Controls were +/+ at the *sg* locus and were recognized as such by a genetic coat-color marker.

Biochemical measurements

Tissue dissection

All animals were anesthetized with Nembutal (40 mg/kg) and were perfused through the heart with 0.1 M PBS maintained at 4°C. The eyes were enucleated; the retinas were removed, and any adhering vitreous humor or pigment epithelium was removed. The olfactory bulbs were removed from the intact brain. All other assays were performed on tissue samples obtained by freehand dissection from ca. 20 coronal 420-μm-thick slices of cold-hardened and agar-encased brains cut with a tissue sectioner (Zigmond and Ben-Ari, 1976). The tissue slices were kept cold throughout the dissection by placing them on a cooling stage mounted on a stereomicroscope. The face of each transverse slice was viewed with intense scattered illumination from below so that individual tissue components could be recognized by their differential translucency (see Fig. 1). Standard dissection protocols for the slices were established in preliminary experiments. As nearly as possible, the entire region to be examined was removed from all slices in which it appeared. Samples from the right and left sides of the brain were pooled after preliminary experiments established that there were no left/right differences in catecholamine content. The dissections were carried out as follows.

Frontal cortex. Samples were taken from slices 1–4 (Fig. 1) and included all of the frontal pole and medial and lateral frontal cortex as far caudal as the anterior extreme of the striatum. Great care was taken not to involve any striatal tissue in these samples, which therefore did not include the caudal limits of medial frontal cortex abutting the rostral pole of the caudoputamen.

Olfactory tubercle. Tissue of the olfactory tubercle was dissected from slices 4–6 (Fig. 1) by following the gray matter from the rhinal sulcus medially to near the midline.

Nucleus accumbens. Samples were taken from slices 4–6 (Fig. 1), in which the limits of the nucleus were determined by the band of olfactory radiation fibers ventrally, the striated appearance of the caudoputamen dorsolaterally, and the change in translucency at the accumbens–septal border medially.

Caudoputamen. Samples were taken from slices 5–12 (Fig. 1) and were identified by the densely striated appearance of all but the dorsomedial corner of the caudoputamen. The globus pallidus could be identified by its darker appearance and was not included.

Septum. Samples were taken from slices 6–8 (Fig. 1) and included tissue dorsal to a line running between the ventral tips of the lateral ventricles.

Amygdala. Samples of amygdaloid tissue were dissected from slices 11 and 12 (Fig. 1) and mainly included the dopamine-rich central nucleus, identified as a rounded translucent zone at the foot of the caudoputamen.

Hypothalamus–preoptic area. Paramedian samples were taken from slices 8–13 (Fig. 1). The dorsal limit of each sample was at a level immediately above the dorsal tip of the third ventricle. The lateral limits were along vertical lines lateral to the fornix bundles. An attempt was made not to include the zona incerta in the samples.

Midbrain. Samples were taken from 5 caudal sections at levels corresponding to slices 14–18 of Figure 1. In the most rostral section, the mammillary bodies were detached, but otherwise the brain stem was included in its entirety at each level. The aim was to include as much as possible of cell groups A8, A9, and A10 of Dahlstrom and Fuxe (1964).

Extraction and measurement of dopamine, norepinephrine, and DOPAC

The tissue samples were homogenized in 0.1 M $HClO_4$ containing 1mM sodium metabisulfite and were centrifuged at 15,000 × *g* for 15 min. The pellet was assayed for its protein content by the method of Lowry et al. (1951). The supernatant was stored at −70°C until assays were begun. The catecholamines in the supernatants were isolated on alumina minicolumns and eluted with 0.2 N or 0.4 N $HClO_4$. The catecholamines were then separated by high-performance liquid chromatography using a reversed-phase column and were subsequently quantified by electrochemical detection (Moyer and Jiang, 1978). Recovery of the catecholamines was estimated by the addition of a synthetic catechol substance, 3,4-dihydroxybenzylamine, as an internal standard to each sample before homogenization and by measuring standard solutions of dopamine, norepinephrine, and DOPAC isolated on separate alumina columns. The recovery of the synthetic catechol averaged 65% and was representative of recoveries of both dopamine and norepinephrine but not of DOPAC, which had average recovery of 45%. Statistical analysis was carried out on group means of sample values using a 2-tailed Student's *t* test when comparison of 2 means was involved (e.g., +/wv vs wv/wv). When multiple comparisons were made among +/+, +/wv, +/wv, and wv/wv animals, statistical assessment was made by a 1-way analysis of variance followed by the Newman-Keuls test (Winer, 1971).

Table 1. Content of dopamine and protein in 3 striatal regions and in the midbrain of homozygous normal (+/+), heterozygous weaver (+/wv) and homozygous weaver (wv/wv) mice

	Dopamine (pmol/mg protein)			Protein (mg)		
Region	+/+	+/wv	wv/wv	+/+	+/wv	wv/wv
Caudoputamen	408 ± 17	356 ± 16[a] (87%)	118 ± 6[ab] (29%)	1.28 ± 0.02	1.36 ± 0.04 (106%)	1.10 ± 0.02[ab] (86%)
Olfactory tubercle	393 ± 23	303 ± 15[a] (77%)	205 ± 16[ab] (52%)	0.187 ± 0.006	0.179 ± 0.008 (96%)	0.190 ± 0.001 (102%)
N. accumbens	301 ± 30	357 ± 22 (119%)	325 ± 22 (108%)	0.145 ± 0.011	0.166 ± 0.012 (114%)	0.153 ± 0.014 (106%)
Midbrain	7.0 ± 0.7	7.0 ± 0.3 (100%)	4.8 ± 0.3[ab] (69%)	4.96 ± 0.17	4.90 ± 0.27 (99%)	4.47 ± 0.31 (90%)

Values represent means ± SEM for 4–17 samples. Samples are from animals that ranged in age from 48 to 256 d. The genotype of each animal was assessed by examination of the cerebellum. Protein content of the tissue was measured using the method of Lowry et al., 1951. The numbers in parentheses are the percent of the corresponding value from +/+ animals. Differences between groups were taken as significant at the $p < 0.05$ level as revealed by the Newman-Keuls test following 1-way analysis of variance.

[a] Difference between indicated group and +/+ is significant.

[b] Value for wv/wv is significantly different from +/+ and from +/wv.

Catecholamine histofluorescence

The brains of 5 wv/wv, 5 +/wv, and 4 +/+ mice were prepared for catecholamine histofluorescence according to the method of de la Torre (1980). Brains were removed from mice deeply anesthetized with Nembutal, were rapidly frozen in pulverized dry ice, and were cut in a cryostat maintained at −12°C. Sections were thaw-mounted, briefly exposed to glyoxylic acid, dried, coverslipped, and studied under incident fluorescence illumination with the aid of a Leitz Ploemopak fitted with filter module D.

Results

Mesostriatal dopamine systems in the weaver brain: Dopamine concentrations in the striatum and midbrain

Figure 2 shows the concentrations of dopamine measured in 3 striatal regions and in the midbrain of homozygous weaver mutants ranging in age from 35 d to 18 months. The results are expressed as percentages of values measured in heterozygous littermate controls and are plotted separately for groups of animals taken for study at progressively increasing ages.

In confirmation of our original findings, there was a marked deficit in dopamine in the weaver caudoputamen and a moderate defect in the olfactory tubercle of the weaver mice but no detectable dopamine deficiency in the nucleus accumbens of these mutants (Fig. 2*A*). This pattern of loss was already evident in the youngest mice studied and was maintained at almost exactly the same levels in the older animals. In the 35-d-old weavers, the caudoputamen contained 34% of the control levels of dopamine, the olfactory tubercle 73%, and the nucleus accumbens 103%.

At each age, an abnormally low concentration of dopamine was present also in the midbrain of the weaver animals (approximately 70% of control levels; see Fig. 2*B*). As in the striatum, the defect was present at its full value in 35-d-old animals and did not change in the older animals.

Comparison of dopamine content in the striatum of homozygous normal (+/+), heterozygous weaver (+/wv), and homozygous weaver (wv/wv) mice

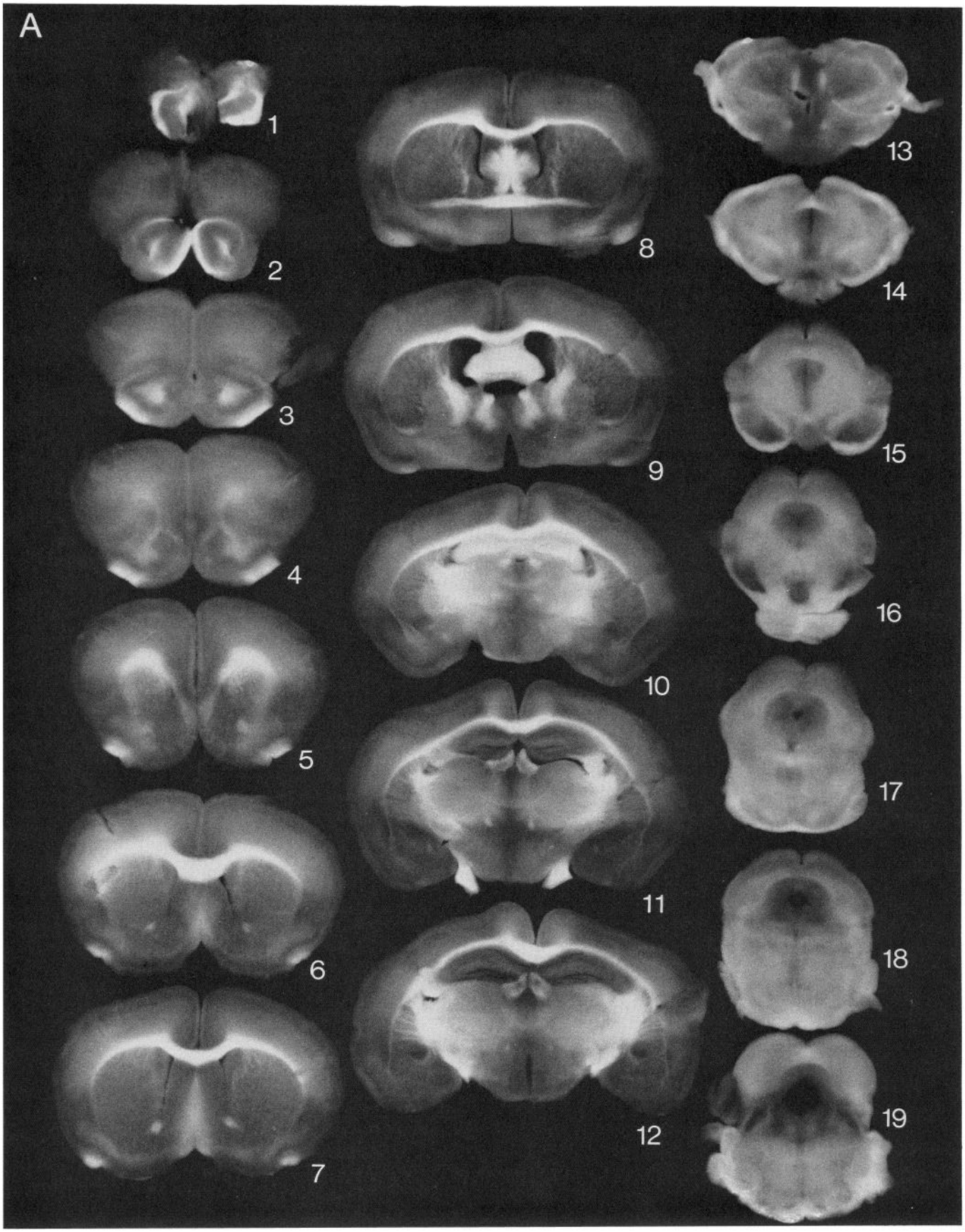
Table 1 summarizes measurements of the content of dopamine in the caudoputamen, olfactory tubercle, and nucleus accumbens from known homozygous normal (+/+) mice, known heterozygous animals (+/wv), and homozygous weavers. Total protein values (Lowry et al., 1951) for each striatal region are also shown. When possible, comparisons were made among littermates of the 3 genetic types; some measurements were carried out on age-matched +/+ mice culled from a colony of +/+ animals maintained on the same (C57BL6/CBA) background. The data from mice of different ages (48–256 d) were pooled, as no age-related differences in the striatal dopamine in weaver mice had been found in our initial study of wv/wv and +/wv controls (Fig. 2).

Clear evidence for a heterozygote effect was evident in the 2 striatal regions vulnerable to a dopamine deficiency in the homozygous weaver mice (Table 1). Relative to the homozygous normal mice, the heterozygotes showed a reduction in dopamine of 13% in the caudoputamen and 23% in the olfactory tubercle. Dopamine loss in the homozygous weaver mutants is accordingly even more severe than originally judged from comparisons with heterozygous controls: In the caudoputamen there was a 71% reduction of dopamine measured against +/+ values (as opposed to 67%); and in the olfactory tubercle there was a 48% loss of dopamine (as opposed to 32%). Values for dopamine in the nucleus accumbens were roughly equivalent in the 3 genetic types. The midbrain of the weaver animals showed a 31% reduction in dopamine content relative to +/+ control valves. There was no significant difference in the dopamine content of midbrain from +/wv and wv/wv animals.

Protein content in the wv/wv animals was different from that in the +/+ and the +/wv animals in the caudoputamen but in no other region examined. A reduction in protein of 14% occurred between wv/wv and +/+ animals. No reduction of protein content was found in the caudoputamen of the heterozygotes relative to +/+ mice. Thus, a heterozygote effect was not present for total protein in the striatum but was apparent for dopamine levels both in the caudoputamen and, most prominently, in the olfactory tubercle.

Histofluorescence in the striatum and midbrain of homozygous normal (+/+), heterozygous weaver (+/wv), and homozygous weaver (wv/wv) mice

Representative cross sections through the striatum of the 3 genetic types are shown in Figure 3. The sections show approximately matched levels through the caudoputamen, nucleus accumbens, and olfactory tubercle. The striking loss of dopamine histofluorescence reported for the caudoputamen of the weaver animals relative to heterozygous controls was readily confirmed, as was the apparent diminution of catecholamine histofluorescence in the lateral part of the olfactory tubercle. As shown in Figure 3*C*, the diminution of fluorescence in the caudoputamen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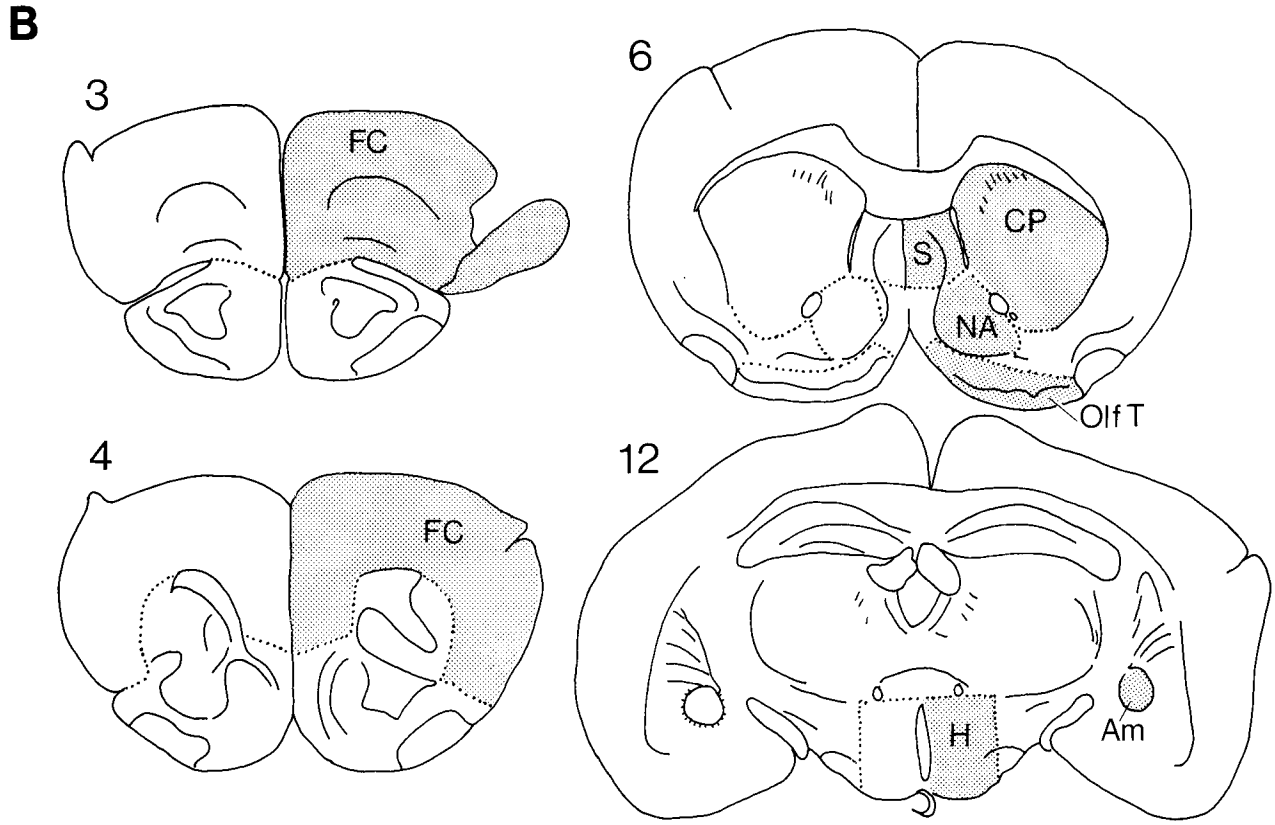

Figure 1. *A*, Photograph illustrating typical series of unstained transverse 420-μm-thick slices prepared for dissection. Standard series contained 19–20 slices. See Materials and Methods for levels from which different brain regions were dissected. *B*, Drawings corresponding to series *3*, *4*, *6*, and *12* of *A* illustrating borders of regions selected for dissection. *FC*, prefrontal cortex; *CP*, caudoputamen; *S*, septum; *NA*, nucleus accumbens; *Olf T*, olfactory tubercle; *H*, hypothalamus; *Am*, amygdala.

affected especially its dorsolateral part. No quantitative assessment of the fluorescence was carried out, but patterns of histofluorescence visible were consistent with a partial loss of dopamine in the olfactory tubercle of the weaver heterozygote relative to the homozygote's content and also were compatible with the biochemical finding of a partial loss of dopamine in the weaver heterozygotes. Neither in the heterozygote nor in the homozygous weaver did the levels of histofluorescence visible in the nucleus accumbens appear to differ from those of the homozygous controls. Thus, the anatomical patterns were in accord with those indicated by the biochemical assays of dopamine in these 3 striatal districts.

In the midbrain (Fig. 4), fluorescent neurons were abundant in the ventral tegmental area (cell group A10) and in the retrorubral area (cell group A8), and such neurons were also present in the pars compacta of the substantia nigra (cell group A9). No attempt was made to map in detail the locations of surviving midbrain neurons using the histofluorescent material, as this is being done as part of a study of the distribution of tyrosine hydroxylase immunoreactive neurons in the weaver mouse (A. M. Graybiel and S. Roffler-Tarlov, unpublished observations). It is important to note, however, that in the weaver mice, fluorescent cell bodies were found in positions corresponding to each of the 3 midbrain cell groups defined by Dahlstrom and Fuxe, including cell group A9, the source of the main dopamine-containing innervation of the caudoputamen. Photomicrographs of fluorescent neurons in the A9 cell group (substantia nigra, pars compacta) are shown in Figure 4 for wv/wv and +/+ mice. At the level shown, the weaver's substantia nigra seems to contain fewer fluorescent neurons in the ventral part of the pars compacta than does the pars compacta of the homozygous normal mouse. Such a distribution would fit with the anatomical pattern of deficient fluorescence in the weaver's caudoputamen, as the ventral pars compacta is known to project to the dorsal caudoputamen (Fallon and Moore, 1978; Nauta et al., 1978). Detailed serial section analysis is necessary, however, for adequate topographic comparisons, and the histofluorescence material was not suitable for such a study.

Content of dopamine and a metabolite, DOPAC, in the 3 striatal targets of mesotelencephalic fiber systems

The concentrations of dopamine and DOPAC extracted from the caudoputamen, the olfactory tubercle, and the nucleus accumbens were compared for +/+, +/wv, and wv/wv littermates (Table 2). Neither catecholamine was changed in concentration in the nucleus accumbens of +/wv or wv/wv animals relative to +/+ controls. In the caudoputamen and the olfactory tubercle, the decrease in content of DOPAC was proportional to that of dopamine. The mean ratio DOPAC/dopamine was not significantly different for the +/+, +/wv, and wv/wv groups in any of the regions sampled, indicating that the rate of formation and use of residual dopamine is unchanged in the affected striatal regions of the weaver brain.

Dopamine in nonstriatal targets of mesotelencephalic fiber systems

Table 3 summarizes measurements of the content of dopamine in 3 nonstriatal regions of the forebrain: the frontal cortex, a major target of the mesocortical dopamine system; and 2 nonstriatal regions classified as targets of the mesolimbic system, the septum and the amygdaloid complex. Each region was examined in weaver mutants, heterozygous weaver littermates,

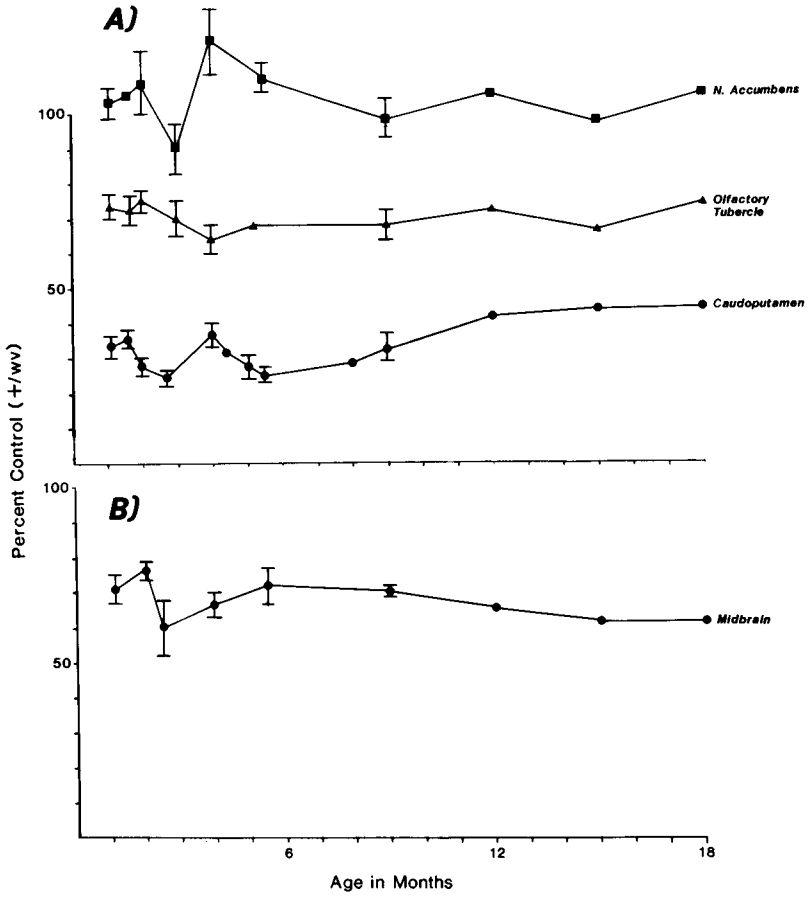

Figure 2. *A,* Dopamine concentrations in 3 striatal regions of weaver mice are expressed as a percentage of values obtained from control heterozygous littermates. Each point is the average of 3–9 animals ± SEM. Where there are no error bars, the point represents the comparison of a single weaver animal with its littermate control. Weavers and littermate control animals were examined at ages ranging from 35 d to 18 months. *B,* The concentration of dopamine in the midbrain of weaver mice ranging in age from 35 d to 18 months is expressed as a percentage of values from heterozygous controls.

and (for the septum) homozygous normal littermates.

In the frontal cortex of the weaver mutants, the concentration of dopamine was low but was not detectably different from that measured in the control mice. Nor was an abnormality in dopamine content apparent in the amygdala, where dopamine levels were high, though still well below (about half) those in the caudoputamen. Endogenous levels of dopamine in the septum were clearly affected in the weaver mutants. As shown in Table 3, there was a 37% reduction in dopamine relative to control values measured in the homozygous normal mice.

Dopamine in regions with intrinsic dopamine-containing cell bodies

The retina, olfactory bulb, and hypothalamus all contain neurons that synthesize dopamine. We previously reported that in the weaver mutant, the content of dopamine in the retina is normal (Roffler-Tarlov and Graybiel, 1984). Measures of retinal dopamine were repeated for the present study, and were again found to be unchanged in the weaver mutants. Assays of dopamine in the weaver olfactory bulb also indicated normal content. However, dopamine extracted from the hypothalamic–preoptic samples in the weaver mice was reduced by 32% relative to heterozygote controls. These findings are summarized in Table 4.

Concentrations of norepinephrine

The content of norepinephrine was measured in all regions in which dopamine levels were measured, excepting only the retina (Table 5). In no region was an abnormality found in the weaver mice. Compared to the high concentrations of dopamine present in the striatum (see Table 2), striatal norepinephrine levels were low. Expressed as a percentage of the dopamine concentrations, the concentrations of norepinephrine were 2% in the caudoputamen, 4% in the olfactory tubercle, and 10% in the nucleus accumbens. As shown in Table 5, the concentrations of norepinephrine in each of these striatal districts and in the septum and midbrain were measured in homozygous normal animals, as well as in heterozygous control and homozygous weaver mutants as part of the study of possible heterozygote effects. No differences were found among the groups.

Dopamine concentrations in the striatum of Purkinje cell degeneration and staggerer mutants

In sharp contrast to the findings in the weaver mice, no deficit in the content of dopamine was present in the striatum of the 2 other types of neurological mutant mice studied (Table 6). The values for concentrations of dopamine in the caudoputamen, olfactory tubercle, and nucleus accumbens in their controls were similar to those found in the weaver controls (compare Tables 1 and 6).

Discussion

Primary and secondary targets of the weaver mutant gene

The actions of mutant genes in the nervous system are both direct and indirect. The cellular target of the direct action of a mutant gene is obliged to suffer its fate regardless of its environment because the genetic defect is intrinsic to the cell itself. By contrast, a cell that is affected as a secondary consequence of the action of a gene can be rescued in altered surroundings. Primary and secondary effects of mutant genes have been identified in several of the neurological mouse mutants. One clear example for the cerebellum occurs in the mutant mouse known as Lurcher, in which virtually all of the cerebellar Purkinje cells degenerate. In the brain stem of this mutant, the numbers of inferior olivary neurons (which have Purkinje cells as their tar-

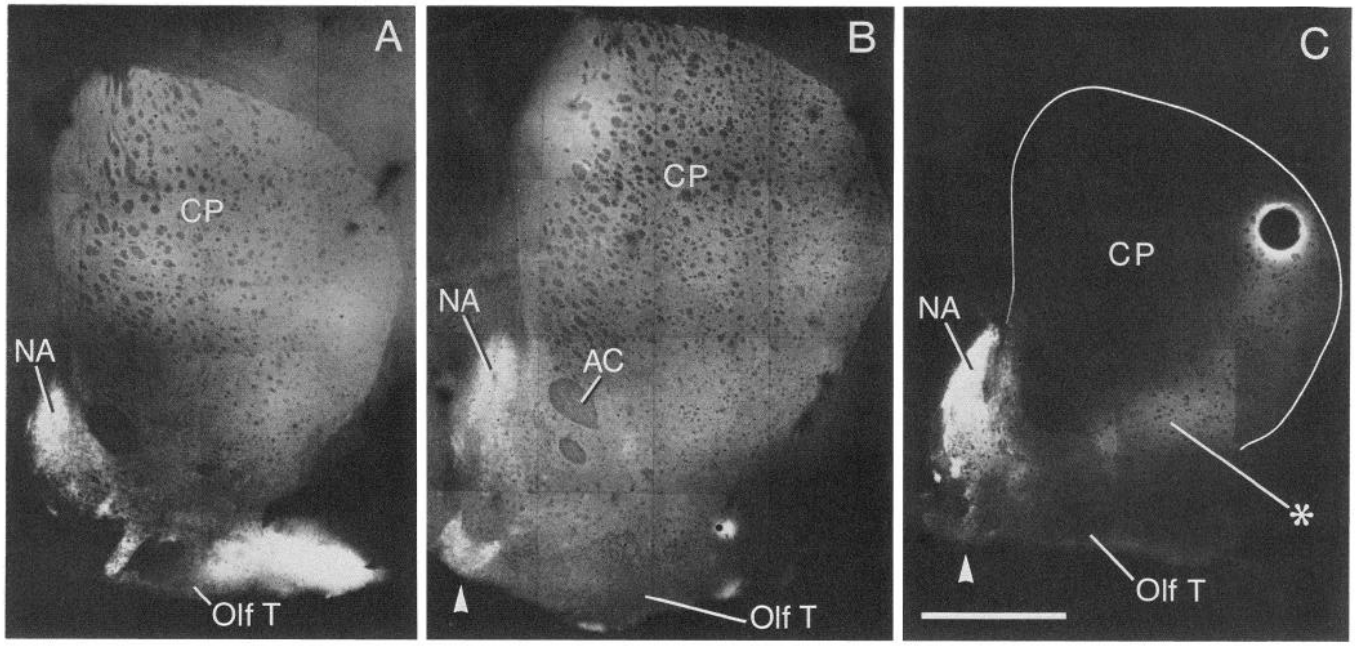

Figure 3. Striatal catecholamine-containing innervation demonstrated in cross sections through the striatal complex of (*A*) homozygous normal (+/+) control, (*B*) heterozygous weaver (+/wv), and (*C*) homozygous weaver (wv/wv) mice. Sections prepared by glyoxylic acid method. The +/+ and wv/wv mice were littermates. The heterozygote (*B*) was an age-matched mouse from another litter. In *C*, small dark circle surrounded by corona is artifact produced at cutting. *White line* has been drawn to delineate caudoputamen medially, dorsally, and laterally. Homozygous normal mouse (*A*) demonstrates densest fluorescence in nucleus accumbens (*NA*) and olfactory tubercle (*Olf T*) and strong but less dense fluorescence in caudoputamen (*CP*). Heterozygote (*B*) shows maintained dense fluorescence in nucleus accumbens and the medial part of the olfactory tubercle (*arrowhead*). Fluorescence in dorsolateral caudoputamen of the heterozygote may be somewhat weaker than that of the homozygous normal, but the difference is not marked. Loss of fluorescence in lateral olfactory tubercle is pronounced. Homozygous weaver striatum (*C*) shows retention of dense fluorescence in nucleus accumbens, some fluorescence in medial part of olfactory tubercle (*arrowhead*), and ventrolateral caudoputamen (*asterisk*). Most of caudoputamen exhibits little fluorescence, the weakest fluorescence being dorsal. *AC*, anterior commissure. Scale bar, 1 mm.

gets) are also severely reduced (Caddy and Biscoe, 1979). When the Lurcher's Purkinje cells are mixed with normal Purkinje cells in the cerebellum of a Lurcher ↔ wild type chimera formed by embryo aggregation techniques, only Purkinje cells carrying the Lurcher phenotype die during subsequent development; the wild-type Purkinje cells are left intact (Wetts and Herrup, 1982). By contrast, inferior olivary neurons carrying the Lurcher phenotype survive in the chimeric inferior olivary complex, which maintains a mosaic of neurons with normal and mutant genotypes (Wetts and Herrup, 1982). The most likely explanation for the rescue of the Lurcher olivary neurons in the chimeric animals is the presence of neuronal targets in the chimeric cerebellum, i.e., the Purkinje cells contributed by the normal component of the chimera. In the Lurcher mutant, the death of olivary neurons is thus probably a secondary epigenetic event, reflecting a loss of such targets, whereas the death of the Purkinje cells is a primary event attributable to the Lurcher gene.

A critical question about the weaver gene has been the identity of the primary cellular target in the cerebellum, where most

Table 2. Content of dopamine (DA) and 3,4-dihydroxyphenylacetic acid (DOPAC) in striatal targets of mesotelencephalic fiber systems (pmol/mg protein)

Structure	DA	DOPAC	DOPAC/DA
Caudoputamen			
+/+	419 ± 19	50 ± 3	0.12 ± 0.006
+/wv	348 ± 14	49 ± 3	0.14 ± 0.008
wv/wv	128 ± 6	20 ± 3	0.16 ± 0.024
Nucleus accumbens			
+/+	334 ± 24	41 ± 6	0.12 ± 0.009
+/wv	378 ± 27	52 ± 2	0.14 ± 0.007
wv/wv	322 ± 20	45 ± 4	0.14 ± 0.003
Olfactory tubercle			
+/+	392 ± 30	39 ± 7	0.10 ± 0.04
+/wv	343 ± 33	35 ± 6	0.10 ± 0.04
wv/wv	156 ± 20	21 ± 3	0.11 ± 0.02

Values represent means ± SEM for 4–9 samples of caudoputamen and nucleus accumbens from animals that ranged in age from 48 to 92 d. The values for olfactory tubercle are from 3–4 samples from animals that were 35 d old.

Table 3. Content of dopamine in nonstriatal targets of mesotelencephalic fiber systems: frontal cortex, amygdala, and septum

	Dopamine (pmol/mg protein)		
Region	+/+	+/wv	wv/wv
Frontal cortex	—	2.2 ± 0.2	2.3 ± 0.3 (105%)
Amygdala	—	158 ± 14	197 ± 26 (125%)
Septum	43 ± 5	40 ± 7	27 ± 1[a] (63%)

Values are means ± SEM of 4–10 samples. Weaver animals were compared with littermate controls; +/wv littermates for frontal cortex and amygdala and both +/+ and +/wv for septum. Frontal cortex was examined in animals between 60 and 270 d of age; septum and amygdala were examined in animals that were 73–195 d old. Numbers in parentheses are the percentage of heterozygous or, in the case of the septum, the homozygous normal control values.

[a] $p < 0.002$.

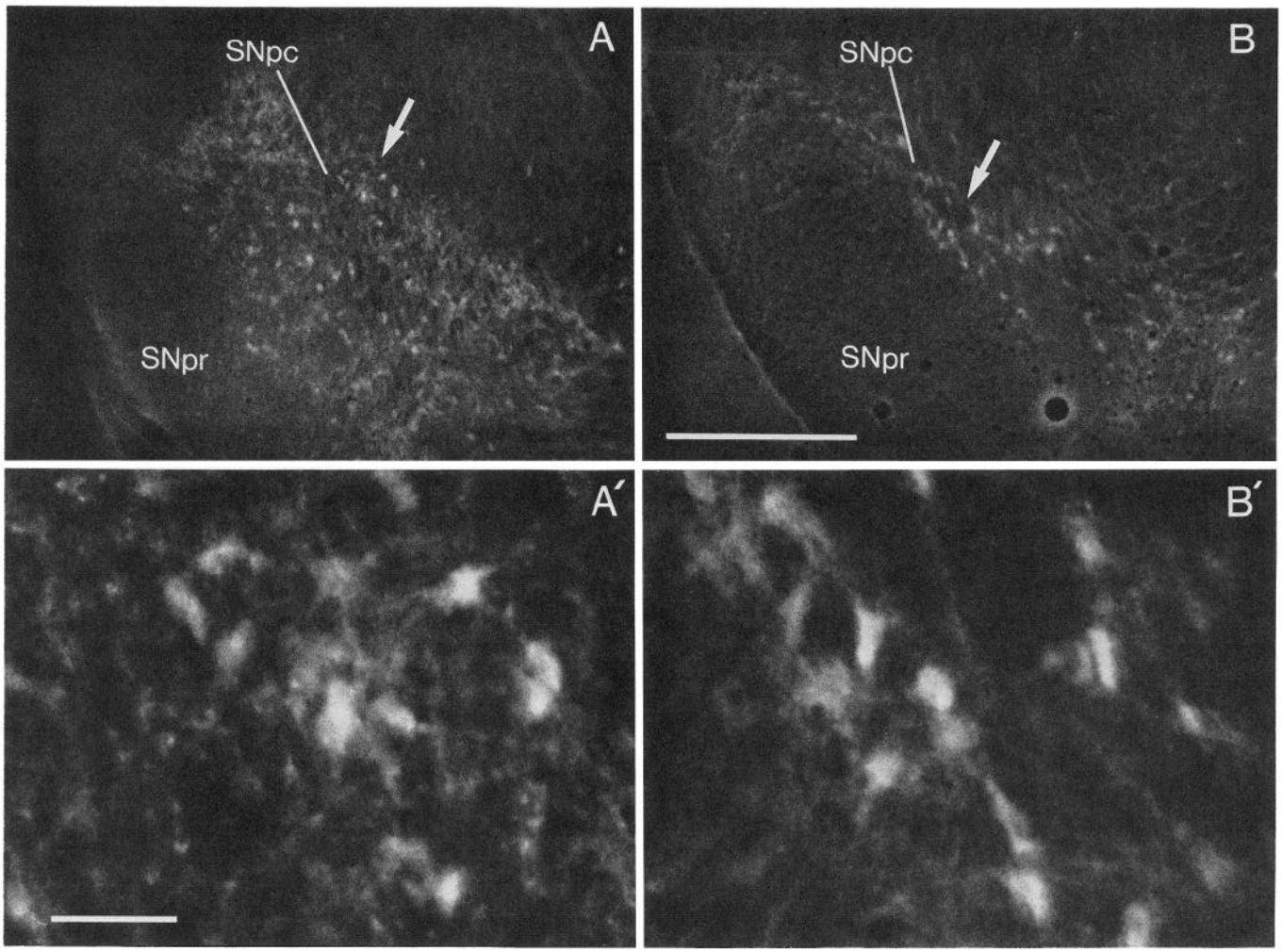

Figure 4. Catecholamine-containing neurons in cross sections through the nigral complex of homozygous normal (*A, A'*) and homozygous weaver (*B, B'*) mice. Sections through the striata of the same mice are shown in Figure 4. *Arrows* in *A* and *B* point to zones shown at higher magnification in *A'* and *B'*, respectively. At the levels shown, there is a paucity of fluorescent neurons in the ventral pars compacta of the weaver relative to the control. *SNpc,* substantia nigra, pars compacta; *SNpr,* substantia nigra, pars reticulata. Scale bars: *A* and *B,* 0.5 mm; *A'* and *B',* 50 μm.

granule cells die, having failed to (1) extend bipolar processes, (2) form specific associations with adjacent Bergman glial guides, and (3) complete successful migration from the external to the internal granule cell layer. The most compelling evidence pointing to the granule cell as a direct target of the weaver gene has come from the study of chimeras formed by fusion of normal (+/+) and heterozygous weaver (+/wv) embryos (Goldowitz and Mullen, 1982). In chimeric cerebella that contained a mixture of heterozygote weaver cells and wild-type cells, the only granule cells found in ectopic positions were those bearing a weaver gene. These cells failed to migrate to the appropriate destination in spite of the presence of normal Bergman glia. Experiments on cultured weaver cerebellar cells support the hypothesis that the weaver defect is intrinsic to the granule cell (Hatten et al., 1984a, b; Willinger and Margolis, 1984a, b), though they also demonstrate an interdependence of young granule cells and Bergman glial cells (see below). None of these studies, including those of the chimeric mice, has eliminated the Bergman glia as being an additional primary target of the weaver gene.

Table 4. Content of dopamine in 3 forebrain regions containing intrinsic neurons that synthesize dopamine: retina, hypothalamus, and olfactory bulb

Region	Dopamine (pmol/mg protein)	
	+/wv	wv/wv
Retina	8.9 ± 0.5	8.3 ± 0.5 (93%)
Olfactory bulb	12.2 ± 0.1	11.7 ± 1.3 (96%)
Hypothalamus	66.9 ± 7.4	45.9 ± 5.4[a] (68%)

Values represent means ± SEM of 5–10 samples. Weaver animals were compared with heterozygous littermates. Olfactory bulbs were examined in animals 48–74 d of age, retinas were from animals 65–142 d old, and hypothalami were from animals 73–188 d old. Numbers in parentheses are percent of heterozygous control littermate values.

[a] $p < 0.05$.

The cerebellum and the mesencephalic dopamine-containing neurons are not linked by direct pathways, so that the abnormalities in the weaver's dopamine-containing systems are certainly not easily attributable to secondary effects of the cerebellar pathology. The findings presented here strongly suggest that the weaver mutation affects a subset of dopamine-containing neurons in the midbrain and that these neurons either comprise a primary site of action of the weaver gene or are intimately

Table 5. Content of norepinephrine in the targets of the mesotelencephalic dopamine-containing systems and the midbrain, the olfactory bulb, and the hypothalamus

Region	Norepinephrine (pmol/mg protein)		
	+/+	+/wv	wv/wv
Caudoputamen	7.7 ± 0.6	7.3 ± 3	7.4 ± 0.8
Olfactory tubercle	16 ± 1	12 ± 1	13 ± 1
Nucleus accumbens	31 ± 3	30 ± 1	29 ± 2
Septum	38 ± 3	44 ± 4	48 ± 4
Amygdala	—	43 ± 4	57 ± 9
Frontal cortex	—	25 ± 1	28 ± 2
Midbrain	36 ± 4	41 ± 2	41 ± 4
Olfactory bulb	—	19 ± 2	17 ± 2
Hypothalamus	—	215 ± 5	266 ± 15

Values are mean ± SEM of 3–17 samples. The animals used ranged in age from 1 to 6 months.

Table 6. Content of dopamine in 3 striatal regions of 2 ataxic mutant mice: staggerer and Purkinje cell degeneration (pcd)

Region	Dopamine (pmol/mg protein)			
	Control	Staggerer	Control	pcd
Caudoputamen	466 ± 17	460 ± 16	403 ± 12	405 ± 18
Olfactory tubercle	247 ± 14	217 ± 25	304 ± 19	316 ± 17
Nucleus accumbens	341 ± 14	366 ± 9	335 ± 18	316 ± 20

Values represent means ± SEM of 4–8 samples. Staggerer and control animals ranged in age between 1 and 14 months. Control animals for the staggerer mutants were littermates or aged matched animals that were +/+ at the sg locus. Controls for the pcd/pcd animals were littermates that could have been +/+ or +/pcd animals. These mice were 6 months old.

connected with such a site. The deficit in dopamine found in the weavers was remarkable for its clean and stable boundaries, reproduced in detail in each animal but appearing only in the weaver mutants among the group of 3 ataxic mutants studied. The fact that neither the staggerer nor the Purkinje cell degeneration mouse mutations showed alterations in striatal dopamine eliminates ataxic movement and cerebellar neuropathology as direct causes of the dopamine depletion in the weaver striatum, and also shows that mutations affecting the cerebellum do not necessarily also affect the striatum.

The best evidence that the reduction of dopamine in weaver is related to a primary site of the action of the weaver gene was the finding of a dose-dependent effect of the gene. In both the caudoputamen and the olfactory tubercle, the 2 striatal regions in which we have found dopamine to be deficient in the homozygous weaver animals, a less marked but significant defect in dopamine was present in the nonataxic heterozygous weaver mice. Gene dose-dependence has already been demonstrated for action of the weaver gene in the cerebellum. A slowed rate of migration of granule cells occurs in the heterozygous weaver cerebellum (Rezai and Yoon, 1972), and the heterozygote suffers a modest loss of granule cells and some disorganization of laminar structure (Rakic and Sidman, 1973a–c). Cultured heterozygous granule cells show abnormal neurite growth (Willinger and Margolis, 1985a, b) and reduced viability (Hatten et al., 1984a; Willinger and Margolis, 1985a, b). The shape, wet weight, content of protein and neurotransmitter-related compounds of the heterozygous weaver cerebellum (Rakic and Sidman, 1973a–c; Roffler-Tarlov and Turey, 1982), and the tyrosine hydroxylase activity of the locus coeruleus (Black, 1976b) are also distinguishable from those of both the homozygous normal and the homozygous weaver cerebellum. Our finding of similar dose-dependent effects on dopamine-containing systems in weaver suggests that the primary actions of the weaver gene are exerted on at least 2 widely separated sites in the weaver brain, viz., the cerebellum and the substantia nigra. It is of interest that concomitant degenerative changes in these 2 structures occur in hereditary diseases in the human; for example, familial olivopontocerebellar degeneration (Jellinger, 1968) and striatonigral degeneration (Rosenberg et al., 1978).

Range of action of the weaver mutation in the forebrain and midbrain

The survey of regions affected and spared in the weaver brains gave clues to the dopamine-containing subsystems vulnerable to the effects of the mutation. First, there was clear evidence for a loss of dopamine in the midbrain. The magnitude of the defect, amounting to about 30% relative to heterozygous controls and homozygous normals, was similar to that measured in our original study of dopamine in the weaver brain (Roffler-Tarlov and Graybiel, 1984). Schmidt et al. (1982) did not observe decreased levels of dopamine in whole brain stem samples from weavers, and Black (1976a) found normal activity of tyrosine hydroxylase in samples of substantia nigra from weaver mice. Schmidt et al. (1982) did report a loss of neurons in the substantia nigra pars compacta of the mutants. Such a loss is consonant with our biochemical findings. Preliminary immunohistochemical experiments suggest a partial reduction of tyrosine hydroxylase-positive neurons in the pars compacta of the weaver's substantia nigra in sections reacted with antibodies to this enzyme (A. M. Graybiel and S. Roffler-Tarlov, unpublished observations). It remains to be determined whether this deficit reflects a loss of neurons or whether neurons are present but elude detection because they do not express tyrosine hydroxylase in appreciable amounts. It is not yet clear whether there is a loss of tyrosine hydroxylase-positive neurons elsewhere in the dopamine-containing cell groups of the midbrain. Strain differences in the numbers of mesencephalic neurons expressing tyrosine hydroxylase have been observed in quantitative studies of such neurons in inbred mice (Baker et al., 1980). The numbers of tyrosine hydroxylase-positive neurons in restricted parts of the substantia nigra, pars compacta, and ventral tegmental area of the BALB/cJ and CBA/J strains differ by about 20%.

For the striatum, our findings confirmed a selective sparing of dopamine in the nucleus accumbens, which receives its dopamine-containing input from the ventral tegmental area and the adjoining medial part of the pars compacta of the substantia nigra. The concentration of dopamine in the olfactory tubercle, already demonstrated to be deficient in the weaver mice compared to heterozygote controls, was shown to be even more significantly affected when the homozygous mutants were compared to homozygous normal mice on the same background. These measurements received support from the anatomical findings. The presence of intense fluorescence in the nucleus accumbens and in medial regions of olfactory tubercle of the weaver mutants cannot be attributed to an increased content of norepinephrine because the content of this transmitter was unchanged in both structures.

The clear-cut differences in vulnerability between dopamine in the nucleus accumbens and in the olfactory tubercle favor the view that these structures should continue to be considered as distinct elements of the basal forebrain despite the fact that they both receive a mesolimbic dopamine innervation and that they are included together as the main components of the ventral striatum (Heimer and Wilson, 1975). The histofluorescence sections further suggest a differential vulnerability of the lateral part of the olfactory tubercle in the weaver animal. This pattern may reflect greater innervation of the medial than the lateral part by particular neurons of the ventral tegmental area and

dorsomedial part of the adjoining pars compacta of the substantia nigra. In the rat at least, cholecystokinin-immunoreactive fibers are especially concentrated in the medial part of the olfactory tubercle (Fallon et al., 1983; Hökfelt et al., 1980a, b), and these fibers are thought to originate in the ventral tegmental–nigral complex (Hökfelt et al., 1980a, b; Skirboll et al., 1981). The medial part of the olfactory tubercle is also thought to receive mainly the "dotted" type of dopamine-containing afferents, rather than the "diffuse" type—so named by Fuxe et al. (1983) for their appearance in sections prepared for formaldehyde-induced fluorescence (Fuxe et al., 1979, 1983). Given the present finding that some fluorescence persists in the medial part of the olfactory tubercle in the heterozygous and homozygous weavers, one possibility is that the weaver mutation particularly affects the diffuse as opposed to the dotted (possibly cholecystokinin-containing) type of dopamine-containing afferents innervating the olfactory tubercle. Alternatively, the dotted type may have a higher terminal dopamine content, so that comparable deficits produce apparent selective sparing.

Of other regions receiving a mesotelencephalic dopamine-containing innervation, only the septum was found to have reduced dopamine in the weaver mutants. The septal region receives dopamine-containing afferents from 2 sources. A mesencephalic innervation arises from a localized paramedian region at the border between the pars compacta of the substantia nigra and the ventral tegmental area (Fallon, 1981; Fallon and Moore, 1978). A similar region is reported to innervate the medial caudoputamen, which was also severely affected in the weaver mice. According to Fallon (1981), the ventral tegmental neurons innervating the septum do not provide a strong collateral innervation of the medial caudoputamen, but some medially situated pars compacta neurons do send collaterals to both regions (and to prefrontal cortex). The second dopamine-containing innervation of the septum arises from cells of the incertohypothalamic dopamine system (Bjorklund and Lindvall, 1978). We could not distinguish between these mesencephalic and diencephalic cell groups as being responsible for the deficit in septal dopamine in the weaver mice. Indeed, the levels in the septum, hypothalamus–preoptic area, and midbrain were all reduced by the same amount (ca. 30%) relative to heterozygote controls.

The 30% deficit in dopamine in the hypothalamus of the weaver was the only finding suggesting that the weaver mutation may have affected dopamine-containing cell bodies other than those of the midbrain cell groups A8–A10. The normal concentration of dopamine was present in the retina and the olfactory bulb, the 2 other regions with dopamine-containing neurons analyzed. The decrease measured in the hypothalamus is difficult to interpret, however, because as much as 40% of the dopamine in the hypothalamus is thought to originate in the midbrain, in the ventral tegmental area (see Kizer et al., 1976). Furthermore, the hypothalamic dopamine-containing neurons are not easily dissected away from those in the zona incerta (cell group A13) or from the rostral pole of the mesencephalic dopamine-containing cell complex. We made an effort to exclude cell group 13 from the hypothalamic samples but were uncertain whether the caudal hypothalamic cell group All was included in the hypothalamic or in the midbrain samples (or in both).

The findings for the frontal cortex merit special comment. Our biochemical measurements failed to demonstrate a loss of dopamine in the frontal cortex of the weaver mutants. Schmidt et al. (1982) did find a reduction of dopamine in their samples of frontal cortex and, in fact, reported as large a deficit in this target of the mesocortical system (70%) as in the caudoputamen. We have no ready explanation for the difference between these findings and our own. Details of the protocols for dissection, however, could have critically affected the values obtained. In our own dissections, we removed frontal cortex only from the 4 slices anterior to the rostral pole of the caudoputamen because our first priority was to avoid involvement of the striatum in the frontal cortex samples. We therefore did not sample all of the rostral cortex innervated by the mesocortical system. For example, little of the anterior cingulate area was included (Bjorkland and Lindvall, 1978; Fallon, 1981). In the pregenual frontal tissue analyzed, however, the findings were unequivocal in suggesting sparing of the mesocortical projection.

Taken together, our findings suggest a remarkable specificity in the dopamine-containing systems affected by the weaver mutation. Of regions innervated by mesencephalic cell groups A8–A10, the nucleus accumbens, amygdala, and prefrontal cortex appear to be spared; but the caudoputamen, olfactory tubercle, septum, and hypothalamus appear to be affected. No single known functional or anatomical category adequately embraces all of the members of either group of structures. On the other hand, certain functional and anatomical dividing lines—for example, between the caudoputamen and the nucleus accumbens—were closely reflected in the pattern of genetic loss. An important generalization suggested by the present findings is that a genetic defect can affect highly restricted parts of the dopamine-containing innervation of the forebrain. By extension, it seems plausible that in other heritable diseases, dopamine concentrations in the frontal cortex, in the nucleus accumbens, or in the amygdala might be affected. Notable in this context is evidence for abnormalities in the dopamine content of the frontal cortex and the amygdala in postmortem brain samples from patients diagnosed as schizophrenics (Bird et al., 1979; Reynolds, 1983).

Our findings are incomplete in that we did not sample all regions receiving a dopamine-containing innervation. For example, we did not include the bed nucleus of the stria terminalis, nor the anterior olfactory area, piriform cortex and entorhinal area, nor the zona incerta. The fact that dopamine was affected in a range of the structures sampled in the weaver brain, not only in the caudoputamen and in the midbrain, tends to point to the dopamine-containing neurons in the brain stem, as opposed to their postsynaptic receptor sites in the forebrain, as being first affected in the progression of the weaver disease.

Biochemical specificity of the genetic defect

The weaver's defect was specific for dopamine among catecholamines. This conclusion was drawn by Schmidt et al. (1982) and was confirmed in the present study for all regions examined, regardless of whether the regions had high or low concentrations of norepinephrine and whether the region was affected or spared in terms of its dopamine content. The fact that norepinephrine content is unaltered by the mutation also indicates that the pool of dopamine available as a precursor in the synthetic pathway for norepinephrine is unaffected.

Comparisons of the concentrations of dopamine and its metabolite DOPAC showed no convincing increase in the content of the metabolite relative to that of dopamine in any striatal region of weaver homozygotes or heterozygotes. This result stands in marked contrast to that obtained after subtotal neurotoxin-induced destruction of the dopamine innervation of the striatum. After the administration of 6-hydroxydopamine, striatal dopamine is permanently decreased and the content of DOPAC is increased relative to the content of dopamine (Zigmond et al., 1984). This result is interpreted as indicating a compensatory increased synthetic activity on the part of surviving dopamine-containing neurons. No such compensatory mechanisms were evident in the weaver's striatum. The dissimilarity may point to a fundamental difference in the response of residual dopamine-containing neurons following an aggressive insult produced by neurotoxins and following a more slowly evolving (and in weaver, genetically determined) process.

The dopamine defect in weaver: parallels with the cerebellar defect

The weaver disease is a consequence of the action of a single gene. Thus, the genetic events that result in the pathologies expressed in the dopamine-containing regions of the brain and in the cerebellum must be caused by a common molecular mechanism. Although the immediate cellular events underlying these pathologies could be quite different, knowledge of the mutation as it is expressed in the cerebellum may nevertheless inform work focused on the dopamine-containing systems. Three key characteristics of the defect in the weaver cerebellum are noted here:

1. The disease as expressed in the cerebellum is a developmental disorder with a visible onset in the early postnatal period. It produces a gene dose-dependent impairment as discussed above, and regional variations in degree of expression occur in different parts of the cerebellum (Herrup and Trenkner, 1985). Similarly, the full deficit in dopamine is present by the end of the first month of life, does not increase with advancing age, shows sharp regional specificity, and reflects a failure of normal maturational increases in the dopamine content of the affected striatal regions (Roffler-Tarlov and Graybiel, 1985a; Schmidt et al., 1982).

2. The weaver gene mutation has a primary neuronal target in the cerebellum—the granule cell. For the dopamine system, it seems likely that a subset of neurons in the midbrain (and possibly in the hypothalamus) are the primary neuronal targets. Similarities between the dopamine-containing neurons and cerebellar granule cells are not obvious, however. They have different perikaryal, dendritic, and axonal morphologies; they express different neurotransmitters; and they arise from different parts of the germinal epithelium during embryogenesis (see Marchand and Poirier, 1983). The critical commonality is likely to be subtle.

3. The death of the cerebellar granule cells in weaver mutants is thought to result from their failure to migrate from the site of their birth in the external granular layer to the internal granular layer (Rezai and Yoon, 1972). Interactions between the granule cells and Bergman glial guide fibers have been implicated in this pathological process. Rakic and Sidman (1973a–c) concluded that the failure of granule cell migration was secondary to defects in the Bergman glia, which in weaver mice are stunted, reduced in number, and unevenly spaced (Bignami and Dahl, 1974). An effect of the mutant gene on these glial cells has neither been proven nor thoroughly discounted, but subsequent work has shown that the fate of the granule cells is not fully dependent on the Bergman glia: Some granule cells die after successful migration to the internal granule cell layer (Sotelo and Changeux, 1974). The nature of the interaction between granule cells and Bergman glial has been probed using young cultured cerebellar cells. The weaver granule cell does not make a close contact with the Bergman glial guide fibers even when cocultured with genetically normal Bergman glial cells (Hatten et al., 1984a, b). Thus, cell–cell interactions apparently necessary for migration of the granule cells are aberrant in the weaver cerebellum.

Given this evidence, it is appealing to think that the defect in the dopamine-containing fiber systems in the weaver brain may also result from abnormal patterns of interaction among neurons destined to synthesize dopamine and other cells, perhaps glial cells. A common molecular mechanism would thus be expressed also in common phenomenological features during the progression of the pathologies. However, the timing of events could not be similar for the cerebellar and dopamine systems if a migratory defect is at issue. The postnatal time of migration of cerebellar granule cells is exceptional. There is no evidence that neurons of the nigral complex undergo major waves of migration postnatally. According to Marchand and Poirier (1983), they migrate in medial and lateral groups during embryonic days E15–18. However, other postnatal defects in cell–cell interactions could lead to the dopamine defect in the weaver brain. For example, neurite extension and guidance both require such interactions (Edelman, 1984). Especially pertinent here are the findings of Denis-Donini et al. (1984) and Hemmendinger et al. (1981), who have found that extrinsic signals influence the outgrowth of neurites by mesencephalic neurons cultured from normal mice. Abnormal growth signals or abnormal responses to such signals might result in defects in neurite formation in the weaver midbrain, with or without subsequent death of the neurons. Just such defects have been found in weaver cerebellar granule cells grown in culture. Relative to normal granule cells, the mutant neurons show an increased frequency of neurite retraction, a markedly slowed rate of growth cone elongation, and shortened neurite length (Willinger and Margolis, 1985a, b).

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