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The Weaver Mutant Mouse as a Model of Nigrostriatal Dysfunction

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Abstract

The weaver mutant mouse has a genetic defect that results in the loss of dopamine neurons in the nigrostriatal pathway. Striatal tyrosine hydroxylase and dopamine content are reduced by 60–70%, and dopamine uptake is reduced by as much as 95%. Deficits in all three of these striatal dopamine markers are seen as early as postnatal d 3. The striatal dopamine systems in the weaver apparently have the ability to compensate for this dopamine deficit. Thus, in the weaver, in vitro resting release, as well as amphetamine-evoked fractional release of endogenous dopamine are increased. An additional change seen in the weaver striatum is an elevated serotonin content. These alterations may play an adaptive role in attempting to compensate for the dopamine loss. In summary, the weaver mutant mouse has dramatic deficits in the nigrostriatal pathway, but also seems to develop certain adaptive mechanisms in dopaminergic and other transmitter systems that may compensate functionally for the dopamine deficit. Thus, the weaver mouse provides a unique animal model for studying naturally induced neuronal degeneration that complements those models using surgical and pharmacological protocols.

Index Entries: Striatum; dopamine; weaver; mouse; neuronal degeneration.

Introduction

The weaver mutation in the mouse is autosomal recessive, and results in abnormal behavior characterized by weakness, hypotonia, tremor, poor limb coordination, instability of gait, and a hind-paw clasping reflex. The most significant features of the neuropathologic phenotype of the homozygous weaver mouse (*wv/wv*) are the severe loss of cerebellar granule cells *(1)* and of midbrain dopamine neurons *(2–5)*. Weaver mice have a dramatic reduction in the number of tyrosine hydroxylase immunolabeled cells in the substantia nigra compared to wild-type control mice *(3–5)*. Consistent with this, *wv/wv* mice also have a reduced content of dopamine in the forebrain *(6)* that is especially severe in the striatum *(2,7)*. In addition, striatal tyrosine hydroxylase activity *(2,8)* and dopamine uptake *(9–11)* are also dramatically decreased. Within the striatum, the dopamine deficit has been shown to be more pronounced in the dorsal than the ventral portion of the nucleus *(7,11)*. Concomitant with the dopamine deficiency, there also exists a behavioral supersensitivity to direct-acting dopamine agonists *(2)* and a biochemical supersensitivity manifested as an upregulation of dopamine D_2 receptors in the

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striatum *(12–14)*. Dysfunction of the striatal dopamine uptake system in the weaver has been observed as early as 7–8 d postnatally despite the finding that striatal dopamine content is normal and the number of TH-positive cells in the substantia nigra was only slightly reduced at this age *(10,15)*. More recent evidence suggests the possibility of degeneration of dopaminergic neurons in the substantia nigra as early as the day of birth *(16)*. In all studies of the weaver mouse at very early postnatal ages, there is a difficulty in identifying the genotype unequivocally, since the behavioral abnormalities characteristic of the homozygous weaver are not readily apparent until about 10 d postnatally. In the preliminary account by Ghetti and Triarhou *(16)*, obligatory weavers were obtained by appropriate breeding methods, thus ensuring that the offspring were homozygous weavers. This may account for the discrepancy between the results of that study and those of Roffler-Tarlov et al. *(10,15)*, who noted only a slight reduction in dopaminergic cell number in 7–8-d-old weaver mice.

In an attempt to gain more detailed information regarding the time-course of changes that take place in the weaver mutant mouse, we have studied various dopamine parameters in the striatum of this mutant, and have compared them to age-matched wild-type mice (+/+) from postnatal d 3 to postnatal d 365. In addition, we have been involved in studies aimed at examining functional neurochemistry of striatal dopaminergic systems in an attempt to learn what kind of compensation or adaptation might take place following dopamine depletion in the nigrostriatal pathway. In this regard, we have investigated the endogenous release of dopamine in striatal slices and have begun an examination of alterations in the striatal serotonergic system in the weaver. The results of our studies on the time-course of changes in the weaver as well as our investigations into functional aspects of dopamine deficiency are the topics of this article.

Methods

Animals

The mice utilized in this study were obtained from a colony maintained at Indiana University School of Medicine and established from mice originally purchased from Jackson Laboratories (Bar Harbor, ME). The weaver gene is maintained on the B6CBA-$A^{W\text{-}J}/A$ hybrid background. In general, homozygous weaver mutant mice *(wv/wv)* were obtained by breeding pairs of heterozygous mice (*wv*/+); heterozygotes were obtained by breeding *wv/wv* females with +/+ males. Normal control mice were wild-type (+/+) from the same hybrid background. The use of homozygous weaver males in the breeding of obligatory weaver mice is made difficult by the fact that the large majority of weaver males are sterile. In our studies on the time-course of changes in dopamine systems in the striatum, we obtained and identified weaver males that were capable of breeding and, therefore, when mated with *wv/wv* females, they sired obligatory homozygous offsprings. Thus, all data collected at postnatal d 3 (except for 5 out of 14 animals used for dopamine content) and postnatal d 7 were from obligatory *wv/wv* mice. Homozygous weaver mice at 5 and 10 d of age were obtained by breeding *wv/wv* females with *wv*/+ males and identifying the *wv/wv* offspring by histological examination of the cerebella. For our studies on dopamine release, all animals were 3 mo of age. All animal use procedures were conducted in strict accordance with the NIH *Guide to the Care and Use of Laboratory Animals* and were approved by the Indiana University Institutional Animal Care and Use Committee.

Tissue Preparation

In experiments on the time-course of changes in dopamine parameters, mice were decapitated, and the brains removed rapidly and placed on a chilled glass plate on ice. Striata were dissected free from surrounding tissue and homogenized in 0.32*M* sucrose using a glass homogenizer and a Teflon™ pestle. In some experiments where dopamine uptake was not determined, striata were homogenized directly in 0.5*M* $HClO_4$ for determination of dopamine content, or in a 1:1 dilution of TH homogenizing buffer (*see* Tyrosine Hydroxylase) for determination of TH activity.

Dopamine Release

In experiments designed to measure endogenous dopamine release from striatal slices, a superfusion system was used as described *(17)*. Following removal of striatal tissue as described above, samples were chopped into 0.3 × 0.3-mm slices using a McIlwain tissue chopper. Slices were transferred to a Plexiglas™ superfusion chamber and superfused with physiological

buffer at 37°C at a flow rate of 0.25 mL/min. After an 80-min washout period, 10-min baseline fractions were collected; buffer was then changed to one containing either elevated K^+ or amphetamine in order to stimulate release. This was followed by a return to normal buffer. All release samples, as well as the tissue extracted at the end of the experiment, were analyzed by HPLC following alumina extraction of the catecholamines and metabolites *(17)*.

Dopamine Content

Endogenous dopamine was extracted from the striatal sucrose homogenates (in those experiments in which dopamine uptake was measured) or from frozen tissue samples, with 0.5*M* $HClO_4$. Following centrifugation to remove acid-insoluble protein, an aliquot of the clear supernatant was injected onto an HPLC column for separation and quantitation of dopamine *(17)*.

Dopamine Uptake

The uptake of ^{3}H-dopamine was determined using aliquots of the sucrose homogenate as previously described *(11)*. Briefly, the uptake assay was conducted by introducing 50 µL of the striatal homogenate into 450 µL of Krebs phosphate buffer containing 10 n*M* [^{3}H]-dopamine. Following a 2 min incubation at 30°C, samples were returned to an ice-water bath and centrifuged immediately at 6000*g* for 15 min. The resulting pellets were surface-washed with 2 mL ice-cold 0.9% saline, and radioactivity was extracted with 0.5 mL of 0.1*M* NaOH. Uptake blanks consisted of samples incubated under identical conditions, except that they were maintained at 4°C throughout the incubation. Extracted radioactivity was determined by scintillation spectrometry at an efficiency of 55–60%.

Tyrosine Hydroxylase

Aliquots of striatal sucrose homogenates were frozen at –20°C until the time of assay. Frozen samples were thawed and diluted 1:1 with "TH homogenizing buffer" (pH 7.4) consisting of 100 m*M* Tris, 2 m*M* EDTA, and 0.008% Triton X-100 (v/v). Aliquots (6 µL) were incubated at 37°C for 20 min with 4 µL of a buffer substrate mixture containing 450 m*M* MES (pH 6.0), 165 U of catalase, 0.25% Triton X-100, 2.5 m*M* dithioerythretol, 4.75 m*M* ascorbic acid, 2.5 m*M* $6MPH_4$, and 250 µ*M* [^{14}C]-tyrosine. Enzyme activity was determined using a CO_2 trapping method as described *(18)*.

Protein

Protein was determined by the method of Lowry et al. *(19)* using bovine serum albumin as standard.

Statistical Analyses

All data were analyzed by ANOVA. Where necessary, the raw data were transformed to remove correlations between means and standard deviations. Data are presented in their untransformed state for ease of understanding. When the main effects and interactions were significant, post-hoc tests were performed using the LSD test. A value of $p \leq 0.05$ was accepted as significant.

Results

Time Course Studies

The data in Fig. 1 illustrate that high affinity uptake of dopamine was significantly reduced in the striata of *wv/wv* mice compared to +/+ mice at all ages. With the exception of postnatal d 7, dopamine uptake was reduced by 60–75% in the weaver and this defect could be observed as early as 3 d postnatally. At postnatal d 7, uptake in the *wv/wv* was decreased by approx 25% relative to that in +/+ mice. The data presented in Fig. 1 indicate also that in the striata of +/+ mice, dopamine uptake continues to increase until 22 d of age, but in the weaver mutant mouse, dopamine uptake is curtailed by postnatal d 7, no further increase being observed after this age. In fact, striatal uptake of dopamine in the *wv/wv* mouse is actually reduced at 6 mo of age compared to that at 22 d of age (Fig. 1).

Striatal dopamine content was significantly less in *wv/wv* mice compared to +/+ mice at all ages except postnatal d 7 and 10 (Fig. 2). With the exception of the data at postnatal d 7 and 10, the magnitude of the decrease in dopamine content varied from about 40% at 3 d of age, to about 65–70% at 6 and 12 mo of age. These data (Fig. 2) also show that striatal dopamine content continues to increase past 22 d of age in +/+ mice to reach a maximum level by 6 mo of age. In *wv/wv* mice, however, striatal dopamine content reached a maximum level by 22 d of age.

Once again, as was seen with dopamine content, striatal TH activity was significantly less in *wv/wv* mice compared to +/+ mice at all ages except postnatal d 7 and 10 (Fig. 3). The magnitude of the deficit in TH (excluding postnatal d 7 and 10) was approx 40–70%, depending on the age. As with

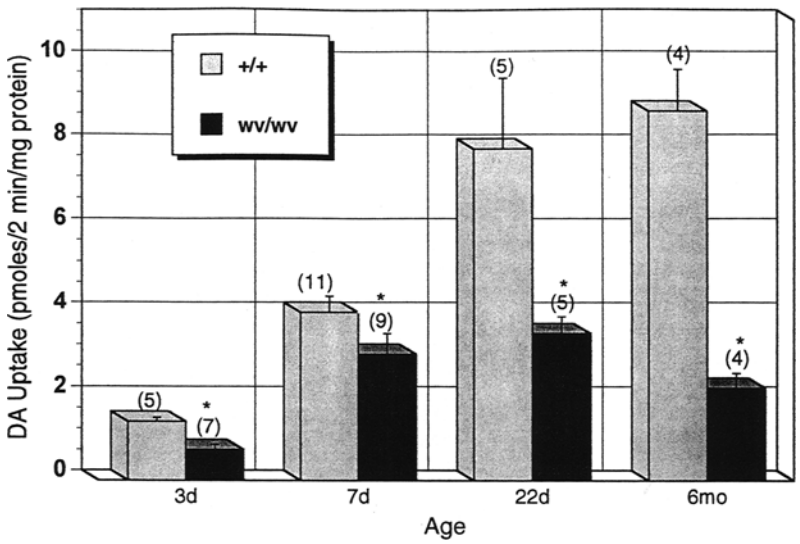

Fig. 1. Age-dependent alterations in dopamine uptake in +/+ and *wv/wv* mice. Dopamine uptake was determined in crude sucrose homogenates at a [^{3}H]-dopamine concentration of 10 n*M*. Values are means ± SEM from the number of determinations indicated in parentheses. *$P < 0.05$ compared to +/+ mice at the same age. Dopamine uptake in +/+ mice as a function of age is 6 mo = 22 d > 7 d > 3 d. Dopamine uptake in *wv/wv* mice as a function of age is 6 mo < 22 d = 7 d > 3 d.

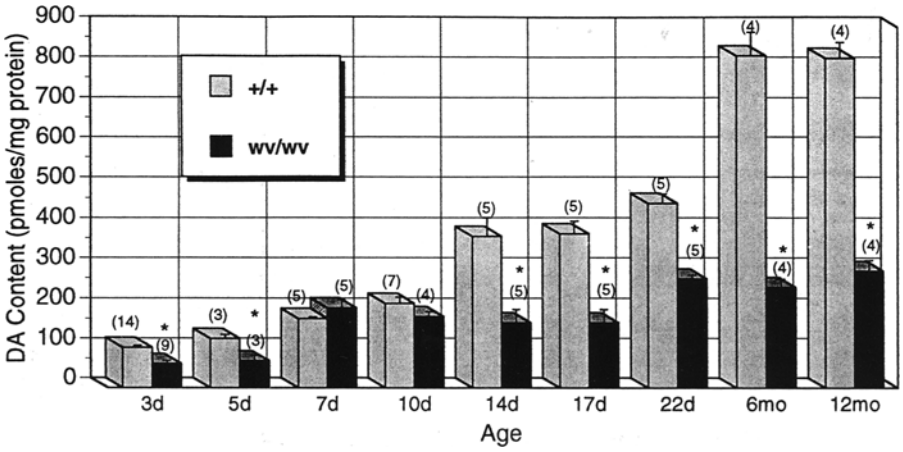

Fig. 2. Age-dependent alterations in striatal dopamine content in +/+ and *wv/wv* mice. Endogenous dopamine content was estimated by HPLC analyses, and the values are presented as means ± SEM from the number of determinations indicated in parentheses. *$P < 0.05$ compared to +/+ mice at the same age. Dopamine content in +/+ mice as a function of age is 12 mo = 6 mo > 22 d = 17 d = 14 d > 10 d = 7 d = 5 d = 3 d. Dopamine content in *wv/wv* mice as a function of age is 12 mo = 6 mo = 22 d > 17 d = 14 d =10 d = 7 d > 5 d = 3 d.

dopamine content, TH activity in the *wv/wv* did not increase past 22 d of age, whereas in the striata of +/+ mice, enzyme activity increased past 22 d of age and reached a maximum by 6 mo of age.

Endogenous Dopamine Release

Resting fractional release of dopamine from striatal slices was 0.24%/min and 0.48%/min for +/+ and weaver mice, respectively (data not shown and ref. *17*), indicating that the weaver releases a greater fraction of the total tissue content than does the +/+ mouse. The data in Fig. 4 show the effect of elevated extracellular potassium and 1 µ*M* amphetamine on dopamine release in the two genotypes. The fractional release elicited by 24 m*M* potassium was not statistically different between weaver and +/+

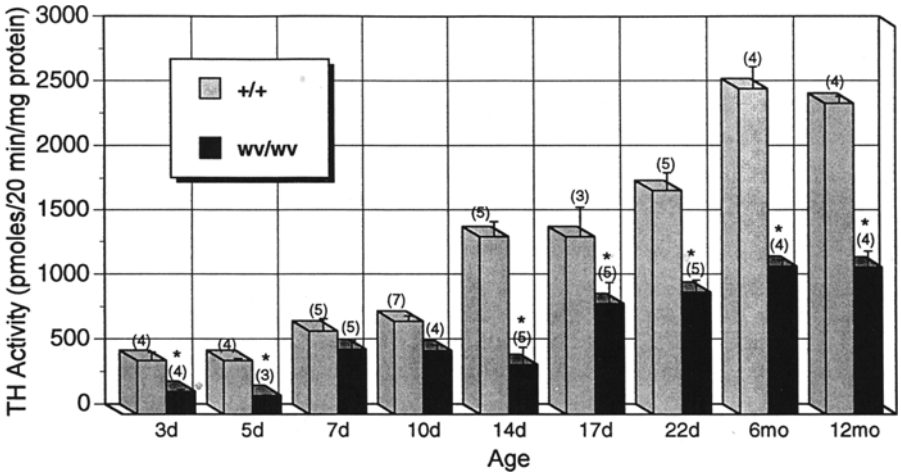

Fig. 3. Age-dependent alterations in tyrosine hydroxylase activity in +/+ and *wv/wv* mice. Tyrosine hydroxylase activity was determined using a CO_2-trapping method, and the values are presented as means ± SEM from the number of determinations indicated in parentheses. Tyrosine hydroxylase activity in +/+ mice as a function of age is 12 mo = 6 mo > 22 d = 17 d = 14 d > 10 d = 7 d = 5 d = 3 d. Tyrosine hydroxylase activity in *wv/wv* mice as a function of age is 12 mo = 6 mo = 22 d = 17 d > 14 d = 10 d = 7 d > 5 d = 3 d.

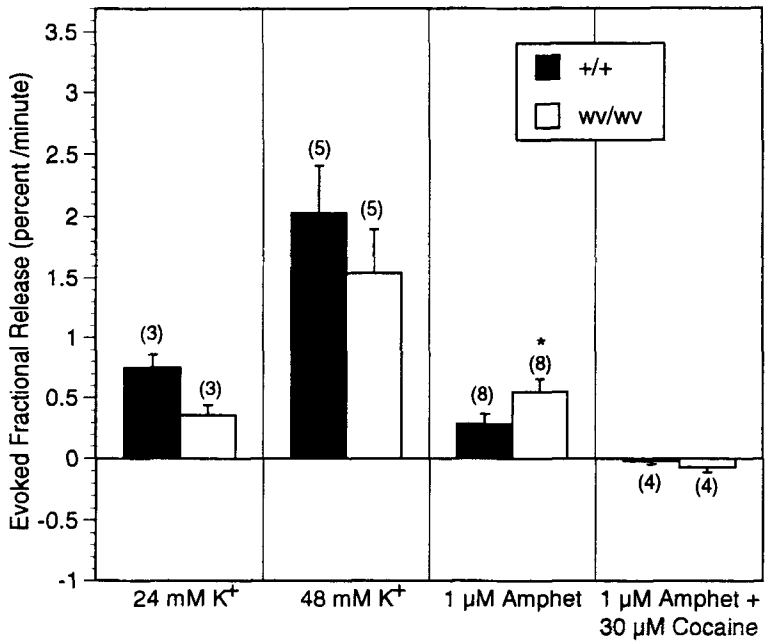

Fig. 4. Evoked fractional release of endogenous dopamine from striatal slices obtained from +/+ and *wv/wv* mice. Evoked fractional release is defined as the stimulated fractional release minus the basal fractional release (*see* ref. *16*). Resting (basal) release for +/+ mice was 0.24 ± 0.03%/min (n = 16) and for *wv/wv* mice was 0.48 ± 0.05%/min (n = 16). Resting fractional release in *wv/wv* mice was statistically different from that in +/+ mice. Values are given as percentage of tissue dopamine released per minute and represent means ± SEM from the number of determinations indicated in parentheses. *$P < 0.05$ compared to +/+ mice (Student's t-test).

mice. Similar observations were made using 48 m*M* potassium. On the other hand, 1 µ*M* amphetamine evoked a significantly greater fractional release from weaver striatum than from +/+ tissue. The amphetamine-evoked release in both genotypes was completely inhibited by cocaine (Fig. 4).

Discussion

Time-Course Studies

The developmental profile for striatal dopamine uptake, content, and tyrosine hydroxylase activity in weaver and wild-type mice is in general agreement with previous reports *(2,10,15)*. We have added important data at postnatal d 3, 5, and 7 using obligatory weaver mice, when possible, to define better the early effects of the *weaver* gene. From these data it is clear that deleterious effects of the homozygous weaver genotype can be seen in the striatum as early as postnatal d 3 when striatal dopamine content, dopamine uptake, and TH activity are all reduced by 40–60% relative to age-matched controls. Such observations might suggest that early effects of the *weaver* gene involve the dopamine terminals in the striatum *(10)* in addition to involvement of the substantia nigra *(5)*. In agreement with the findings of Roffler-Tarlov et al. *(10)*, we also found that the striatal content of dopamine was not different between weaver and +/+ mice at postnatal d 7. In addition, we now also report that tyrosine hydroxylase activity is the same between these two genotypes at d 7, and that there are no differences between the two genotypes at postnatal d 10 regarding tyrosine hydroxylase and dopamine content. Thus, despite the large deficit in dopamine content, dopamine uptake, and TH activity at all the other ages studied, the dopamine content and TH activity in the 7- and 10-d-old weaver striatum appears to be normal, and dopamine uptake (at postnatal d 7) is reduced by only 25% relative to +/+ mice. It is conceivable that in the 7–10-d-old weaver mouse, there might be a burst in the neuronal maturation process or, perhaps, an attenuation of the pruning process, either or both of which would lead to the observed results. However, the reasons for the apparent normalcy of striatal dopaminergic neurons in the weaver mouse at 7 and 10 d of age remain speculative at this time.

The reduction in striatal dopamine uptake in the weaver mouse is not likely to be a result of excessive efflux of dopamine *(17)*, nor is it likely to be a result of an altered velocity of a full complement of transporters, since autoradiographic observations indicate that the number of dopamine transporters is, in fact, reduced in the weaver striatum *(9)*. The most likely explanation for the reduced dopamine uptake in the striatum of the weaver is that there are fewer dopaminergic fibers innervating this region.

Release Studies

The release of dopamine from striatal preparations has been used to gain insight into some of the synaptic functional consequences of the weaver mutation. The observation that fractional resting release is greater in weaver than in +/+ mice suggests the possibility that adaptive mechanisms may take place in the dopamine-deficient striatum of the weaver in order to overcome this deficit. Such an adaptation has been seen in the 6-hydroxydopamine lesioned rat striatum where resting release of striatal dopamine was the same in control and lesioned rats despite a significantly lower tissue content of dopamine in the lesioned animals *(20,21)*.

The possibility that the elevated fractional resting release from the weaver striatum might be the result of the severely compromised uptake system in this mutant *(10,11)* seems unlikely in view of the efficiency of the superfusion system employed. Thus, in studies on basal dopamine release, neither increasing the superfusion rate nor including 30 μ*M* cocaine in the superfusion buffer had any effect on the recovery of released dopamine (data not shown). These findings support the notion that the superfusion was efficient enough to capture most, if not all, of the released dopamine, essentially eliminating reuptake. Similar arguments would also apply in ruling out a possible involvement of the dopamine autoreceptor as being responsible for the elevated fractional release in the weaver *(22–25)*.

Another possibility in explaining the higher fractional resting release of dopamine in the weaver is that tonic influences from other transmitter systems are altered in the *wv/wv* striatum. For example, activation of a tonic excitatory input, or inhibition of a tonic inhibitory input in the weaver would be consistent with an elevated factional resting release compared to that in +/+ mice. Two likely candidates for transmitters that might affect dopamine release in the striatum are serotonin and glutamate *(26,27)*. In fact, we recently reported elevated serotonin content and increased immunocytochemical staining for 5-HT in the striatum of the weaver mouse *(28)*. In addition, activation of serotonin receptors has been shown to increase rat striatal dopamine release *(26)* and in the dopamine-deficient, neonatally lesioned rat striatum (6-OHDA lesion), serotonin hyperinnervation of the striatum has also been reported *(29–34)*. Such possible interactions of other transmitters with the dopamine release mechanism in the striatum might also

explain the differential response of the two genotypes to elevated potassium and amphetamine. Potassium is a nonspecific stimulus and will induce the release of many transmitter substances in the striatum. With the release of multiple transmitters and their potential for modulating dopamine release, it might prove difficult to detect differences in dopamine release between +/+ and weaver mice. Since amphetamine is a more selective releasing agent than is elevated potassium, these confounding factors would be eliminated, thus allowing genotype differences in dopamine release to be detected. Thus, the significant increase in fractional dopamine release induced by amphetamine in the weaver, compared to that seen in the +/+ mouse, suggests a functional difference in the dopamine release mechanisms between the two genotypes.

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