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Reversal by L-Dopa of Impaired Learning Due to Destruction of the Dopaminergic Nigro-Neostriatal Projection

Abstract. *Rats receiving bilateral stereotaxic injections of 6-hydroxydopamine into the zona compacta of the substantia nigra failed to learn a one-way active avoidance response. Small doses of* L-*dopa (1.5 milligrams per kilogram of body weight) in combination with a peripheral decarboxylase inhibitor reversed this impairment. Animals with lesions which acquired the avoidance response during* L-*dopa administration retained this response when drug treatment was discontinued. These experiments suggest that the dopaminergic nigro-neostriatal projection serves a critical function in the acquisition of learned instrumental responses.*

Many pharmacological studies suggest that brain catecholamines (CA) play an important role in the acquisition and maintenance of conditioned avoidance responding (CAR). For example, drugs that deplete central monoamines or block postsynaptic CA receptor sites have repeatedly been shown to disrupt avoidance responding (*1*). In addition, intraventricular application of 6-hydroxydopamine, which can cause substantial and selective destruction of central CA neurons, has been found to produce deficits in CAR (*2*). While several workers have implicated dopamine rather than norepinephrine (NE) as being critically involved in CAR, it has not been possible to evaluate the role of specific CA pathways with these techniques.

With the discovery that specific CA projections could be selectively lesioned by the localized stereotaxic injection of small quantities of 6-hydroxydopamine, new possibilities for the investigation of the functional roles of these systems have emerged (*3*). We have shown that rats with bilateral 6-hydroxydopamine lesions of the zona compacta of the substantia nigra, the origin of the dopaminergic nigro-neostriatal bundle (NSB), will not acquire either a CAR or an instrumental response for food reinforcement (*4*). If, however, the animals were overtrained on CAR prior to the lesions and then subsequently retested, almost perfect retention of the response was observed. Thus the deficits produced by these lesions are not the result of generalized motor deficits and appear to be specific for the acquisition of CAR.

The deficits in CAR produced by reserpine or α-methyl-paratyrosine can be temporarily reversed by the CA precursor, 3,4-dihydroxy-L-phenylalanine (L-dopa) (*5*). Furthermore, L-dopa is known to be clinically effective in the treatment of Parkinson's disease, a condition in which the dopaminergic nigro-neostriatal projection has degenerated (*6*). We now report that the deficits in CAR produced by destruction of the dopaminergic NSB can be reversed by L-dopa treatment in the rat.

Table 1. Effect of bilateral 6-hydroxydopamine lesions of the substantia nigra on brainstem and hypothalamic norepinephrine and striatal dopamine concentrations. Data represent means (± 1 standard error of the mean) of 15 to 18 animals in each group.

Item	Controls (μg/g)	6-Hydroxydopamine (μg/g)	% Control
Striatal dopamine	10.1 ± 0.32	0.84 ± 0.11*	8.3
Hypothalamic NE	1.67 ± 0.04	0.74 ± 0.05*	44.3
Brainstem NE	0.36 ± 0.01	0.46 ± 0.01*	127.8

* Significantly different from controls $P < .01$.

Eighteen male Wistar rats (280 to 320 g) received bilateral stereotaxic 6-hydroxydopamine lesions of the zona compacta of the substantia nigra by a procedure that has produced nearly total destruction of the dopaminergic NSB (*4*, *7*, *8*). Control animals ($N = 16$) underwent sham operations but did not receive intracerebral injections. After the operation the experimental animals became aphagic and adipsic and had to be tube-fed as described (*7*). After the animals with lesions recovered the ability to maintain themselves, they were divided into two groups matched for body weight. The control group was similarly divided. Six weeks after the surgery, all animals were tested for their sensitivity to L-dopa. Previous experiments have demonstrated that rats lesioned with 6-hydroxydopamine show an increased responsiveness to L-dopa, an effect that is presumably mediated by postsynaptic receptor supersensitivity (*9*). The purpose of this procedure was to determine a dose of L-dopa that was just below that which would induce stereotyped behavior. All animals received Ro 4-4602 (50 mg/kg) 30 minutes before they were given injections of L-dopa to inhibit peripheral decarboxylase activity (*10*). Such preliminary treatment enhances the central effects of L-dopa (*11*). L-Dopa at 25 mg/kg administered intraperitoneally produced intense stereotyped behavior in the lesioned animals, but did not have any noticeable effect on the controls. Lower doses (12.5 and 3.0 mg/kg) also induced stereotypy but 1.5 mg/kg did not, and hence this dose was used in the CAR experiments.

The acquisition of a one-way active avoidance response was studied as described (*4*). Briefly, rats were placed in one side of a wooden box (75 by 25 by 30 cm) divided into two equal compartments by a sliding door. The lifting of the door initiated a discrete tone that preceded the delivery of shock (0.7 ma) through the grid floor by 10 seconds. The grid shock lasted for 5 seconds, and in no instance did any animal fail to escape from the shock within that time. The intertrial interval was 30 seconds and was spent in the "safe" compart-

ment. Each animal received ten trials per day for 8 days. Each day, 1 hour before CAR training all the animals received an intraperitoneal injection of Ro 4-4602 (50 mg/kg). Thirty minutes later, half of the lesioned group and half of the controls received intraperitoneal L-dopa (1.5 and 25 mg/kg, respectively) while the other half of each group received injections of the vehicle. After the 8-day acquisition period, the animals were not tested or given drugs for 4 days. On days 13 to 17, the treatments were changed so that animals that had previously received vehicle injections were given L-dopa as described above, while the animals that were treated with L-dopa for 8 days now received vehicle injections before each daily CAR session. The animals were killed 2 weeks after the final CAR session, NE was measured in the hypothalamus and brainstem, and dopamine was measured in the caudate putamen (*12*). The data were assessed statistically by analysis of variance and Student's *t*-test.

Control animals acquired the CAR to a criterion of eight out of ten avoidance responses on two consecutive days by day 4 or 5 (Fig. 1). L-Dopa (25 mg/kg) did not significantly influence the performance of the control animals. As was observed previously, the group receiving bilateral NSB lesions failed to acquire the CAR. Although escape latencies were not formally recorded, they typically occurred 1 to 2 seconds after the onset of footshock. In contrast to the group injected with the vehicle, the nigral animals receiving L-dopa (1.5 mg/kg) acquired the CAR. This group did not perform quite so well as the controls, but this difference was not statistically significant (Fig. 1). When the animals with nigral lesions that had originally received vehicle injections were injected with L-dopa on days 13 to 17, they also acquired the CAR within a few days. Thus, these animals that had performed only one or two avoidance responses out of a possible ten per day for 8 days were making eight or nine daily avoidance responses after a few days on L-dopa. The performance of the nigral group that had received L-dopa during CAR acquisition did not deteriorate when they were changed to vehicle injections on days 13 to 17 (Fig. 1).

The effect of the nigral lesions on the content of dopamine and NE in several regions of the brain are seen in Table 1. Catecholamine levels did not differ significantly between the two nigral

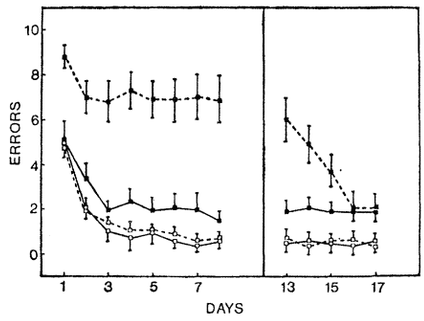

Fig. 1. Effects of L-dopa on the acquisition of a one-way active avoidance response in rats receiving either prior 6-hydroxydopamine lesions of the substantia nigra or sham operations. Each rat was given ten trials per day for 8 days and the number of errors each day (lack of avoidance response) are given on the abscissa. On days 9 to 12, no avoidance tests were conducted. On days 13 to 17, L-dopa administration was reversed so that rats previously receiving vehicle injections were injected with L-dopa, while those given L-dopa on days 1 to 8 received vehicle. All animals were injected intraperitoneally with Ro 4-4602 (50 mg/kg) at 60 minutes and L-dopa or vehicle at 30 minutes before daily avoidance training. Data represent means (± 1 S.E.M.) of eight or nine animals in each group. ■—■, nigral lesions plus L-dopa (1.5 mg/kg); ■ - - ■, nigral lesions plus vehicle; □—□, controls plus L-dopa (25 mg/kg); □ - - □, controls plus vehicle.

lesioned groups (L-dopa and vehicle) and therefore the data from these groups were pooled. The data from the two control groups were pooled for the same reason. Striatal dopamine was reduced to less than 10 percent by the nigral 6-hydroxydopamine injection. Hypothalamic NE was also significantly reduced by this procedure, and this probably reflects damage to the ventral NE bundle which courses just dorsomedial to the zona compacta of the substantia nigra (*3*). Brainstem NE was significantly increased by the 6-hydroxydopamine lesions, however, and this may have been due to either accumulation of NE on the proximal side of lesioned axons or to sprouting of new axon collaterals caudal to the lesion site (*3, 13*).

These data confirm the critical importance of the dopaminergic nigro-neostriatal projection in the acquisition of a CAR. The fact that very small doses of L-dopa can almost completely reverse the learning deficit produced by nigral injections of 6-hydroxydopamine provides strong evidence that the destruction of this dopaminergic system rather than possible nonspecific effects of 6-hydroxydopamine were responsible for the deficits. However, the nigral lesions also produced significant damage to the ventral NE bundle (Table 1). While lesions to this system do not by themselves produce deficits in CAR (*4*), we cannot at present rule out the possibility that deficits in avoidance responding are the result of combined lesions to these two catecholaminergic projections. Earlier we reported that prior overtraining of an avoidance task rendered the animals relatively immune to the disruptive effects of nigral 6-hydroxydopamine lesions on CAR. In the present experiments a similar observation was made insofar as lesioned animals that were trained during L-dopa administration continued to perform adequately on this task when L-dopa was discontinued.

These and earlier results point to the special significance of the dopaminergic nigro-neostriatal projection in the learning of instrumental responses. Although the precise nature of the behavioral deficit produced by lesions of the NSB has not yet been determined, at least three hypotheses deserve consideration. First, the lesioned animals may not be able to learn the significance of the conditioned stimulus and hence do not avoid the ensuing shock. This hypothesis seems unlikely in view of our preliminary observation that identical 6-hydroxydopamine lesions of the substantia nigra do not impair learning in a conditoned suppression paradigm. A second hypothesis is that while the lesioned animals may appreciate the significance of the conditioned stimulus they may not be able to initiate the appropriate motoric response to avoid the shock. That is, nigral lesions may result in a "performance" rather than a "learning" deficit. In this regard, it is significant that one of the cardinal symptoms of Parkinson's disease is the inability to initiate movement (*14*). In addition, the remarkable ability of Parkinsonian patients to temporarily overcome this inability when confronted with strong environmental or emotional stimuli (*14*) may be analogous to the fact that the rats with nigral lesions will escape from but do not avoid foot shock. This second hypothesis does not explain, however, the finding that 6-hydroxydopamine lesions of the substantia nigra impair the acquisition but not the retention of a CAR (*4*). The third alternative relates to a recent observation by Anlezark, Crow, and Greenway (*15*) who reported that bilateral lesions of the locus ceruleus impair the acquisition of a learned response for food reinforcement. Both the dorsal noradrenergic bundle which originates in the locus ceruleus and the

dopaminergic NSB will support intracranial self-stimulation and may therefore play important roles in reinforcement (*16*). It is therefore possible that the learning deficits observed after lesions of these projections may be due to an inability of these animals to be reinforced by correct responses.

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Sex-Associated Antigens in Mice and Rats

Silvers and Yang (*1*) and Wachtel *et al.* (*2*) report on the potency and homology of male-specific antigens as shown by immunization or skin grafting of female recipients by male donors. Readers uninitiated to the field of transplantation immunogenetics may be left with several misleading impressions from the evidence presented. The finding that male lymphoid cells from some but not other strains of rats sensitize C57BL/6 female mice against subsequent C57BL/6 male skin grafts suggests molecular similarity or cross-reactivity between certain male antigens of rats and mice. Actually, male cells from only four of nine rat strains tested were effective in sensitizing female mice against male skin grafts. Moreover, the sensitization achieved was modest and highly variable. Despite this seemingly strong evidence of heterogeneity of male-specific antigens and of responsiveness to such antigens among rats and mice, Silvers and Yang imply that a single Y chromosome determined antigen is involved and is identical in all strains of rats and mice (*1*). Only the conclusion that these mammalian species may share similar H-Y or other male-specific antigens is justified by the evidence.

Wachtel *et al.* (*2*) found that (B10 × B10.BR)F_1 females rejected B10.BR ($H\text{-}2^k$) male grafts faster than ($H\text{-}2^b$) grafts, while F_1 hybrid male grafts yielded an intermediate median survival time. This confirms earlier reports (*3*) that the *H-2* locus itself or closely linked genes moderately influence the degree of responsiveness to male-specific antigen (or antigens). Wachtel *et al.* conclude that the potency or "immunogenicity of H-Y is influenced by the *H-2* locus," but this interpretation is questionable because the congenic strains tested display multiple genetic differences in the H-2 region. The C57BL/10 and B10.BR strains carry different alleles at the *Ir-1* and *Ss-Slp* loci and have significantly different serum complement levels (*4*). Moreover, the *Slp* locus governs a serum globulin antigen restricted to male mice. Much evidence favors the conclusion that *H-2* or associated *Ir* (immune response) genes regulate recipient responsiveness to particular immunogens (*5*). The molecular structure of the H-Y immunogen should remain unchanged, unless antigenic modulation occurs in the foreign environment of the recipient.

The major objection to the male → female experimental design employed by Silvers and colleagues is that one could be dealing with an autosomal gene (or genes) with products sex-limited to males. Moreover, the disparate hormonal environment of males and females affects immune responsiveness as well as gene expression (*6*). Females generally give more vigorous immune responses than males. Actually, male specific cellular antigens of both autosomal and H-Y origins may exist (*7*), a supposition supported by the recent finding of a mouse sperm-specific antigen governed by a *T*-locus gene (*8*). Use of the parental → reciprocal F_1 hybrid male design not only avoids objections to the male → female design, but provides a test for allelism and relative immunogenicity of both *H-X* and *H-Y*. With this approach, we repeatedly found both allelism and nonidentity of products of *H-X* and of *H-Y* loci in mice (*7*, *9*) and in rats (*10*). In our most extensive study in mice (*7*), different X-linked incompatibilities were stronger than the Y-linked ones in relation to otherwise identical male recipients. The existence of multiple *H-X* alleles in mice has been confirmed and extended by D. W. Bailey (*11*), although Barnes (*12*) has not found expected H-X associated skin graft rejection in certain F_3 male → F_1 male hybrid experiments. We noted (*7*), in a report not cited by Silvers *et al.* (*1*), that reactions to these weak sex-associated antigens can be greatly influenced by such variables as prior immunization, graft dosage, and the particular allelic combination. Indeed, "X-Y barriers in these male to male combinations are so weak that many large grafts remained viable for nearly the entire life span of the recipients (*7*, p. 27).

The failure to confirm H-Y allelism, reported by Wachtel *et al.* without supporting data [reference 7 in (*2*)], is hardly acceptable, especially when extensive experiments with numerous controls and subtleties are involved. It is easy to get long-term acceptance of skin grafts across the weak H-Y (or