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# Proposed animal model of severe Parkinson's disease: neonatal 6-hydroxydopamine lesion of dopaminergic innervation of striatum

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**Summary.** Rats lesioned shortly after birth with 6-hydroxydopamine are posed as a near-ideal model of severe Parkinson's disease, because of the non-lethality of the procedure, near-total destruction of nigrostriatal dopaminergic fibers, near-total dopamine (DA)-denervation of striatum, reproducibility of effect, and relative absence of overt behavioral effects – there is no aphasia, no adipsia, and no change in motor activity. In vivo microdialysis findings reinforce the utility of the animal model, clearly demonstrating L-DOPA beneficial actions without an increase in hydroxyl radical production.

## Introduction

An adulthood lesion of either *pars compacta* substantia nigra (pcSN) or nigrostriatal dopaminergic tracts produces a reasonable animal model of Parkinson's disease (PD), but the animal is beset with major behavioral deficits that weaken and threaten survival: aphagia, adipsia, immobility, and lack of grooming. Also, there is often large variation in effect resulting from small differences in positioning of a needle tip or cannula tip (or electrode) in pcSN or along the nigrostriatal tracts (Zigmond and Keefe, 1998).

Breese and colleagues, in an extensive series of studies, found that a 6-hydroxydopamine (6-OHDA) lesion of neonatal rats produced near-total destruction of the nigrostriatal dopaminergic tract (Breese and Breese, 1998). Moreover, gross behavior of the rats was little changed from control – mobility and grooming were unaltered; and there was an absence of aphasia and adipsia. Through a series of large studies in my laboratory – involving more that a thousand rats – we recognize that this preparation is a near-ideal animal model of severe PD. In particular, there is 99% destruction of dopamine (DA) innervation of striatum, the extent of DA neuronal damage is reliable and consistent (∼1% variation of effect) and 99% or the rats survive the procedure. Ongoing *in vivo* microdialysis studies reinforce the view of this preparation as an excellent model of severe PD.

## Methods

At 3 days after birth rat pups are pretreated with desipramine HCl (20 mg/kg IP), 1 h before bilateral ICV administration of 6-hydroxydopamine (6-OHDA; 67 µg, base, on each side) or saline-ascorbic acid (0.1%) vehicle. To administer the latter substances, rat pups are individually immersed in ice (∼60 sec) to produce cold-anesthesia. Pups are then placed on a flat surface under a bright light. In this manner, the sagittal and

transverse sinuses overlying the cranium, as well as bregma and lambda, can be seen through the transparent intact dermis. A 26-gauge needle, attached to a microliter syringe, is positioned 1.5 mm anterior to lambda and 2 mm lateral to the sagittal plane. The needle, equipped with a polyethylene sleeve up to 2 mm from the tip, is then lowered to the stop position (i.e., sleeve), with the needle in the lateral ventricle. After injection of 5 µl of 6-OHDA or vehicle, the needle is left in place for at least 30 sec. Immediately afterward, an injection is made in the same manner into the other lateral ventricle. Pups are warmed to promote recovery, and each pup is then returned to the litter. In adulthood, litters treated with 6-OHDA are generally ~5% smaller than controls – although this can be minimized if desired by exchanging dams (Brus et al., 1994). When these rats are used as a model of PD, there are no additional treatments during development or before the time of the PD study.

## Results

### *Neurochemical and neuroanatomical effects on striatal DA innervation*

In rats treated at 3 days after birth with 6-OHDA, there is an marked reduction in adulthood levels of striatal DA (~99% ± 1%), DOPAC (~99%) and HVA (~99%). Anatomically, there is ~99% reduction in the number of tyrosine hydroxylase (TH) immunopositive fibers innervating striatum. This serves as a near-ideal neurochemical and neuroanatomical template of severe PD (Kostrzewa et al., 1998).

### *General behavior of 6-OHDA-lesioned rats*

Rats lesioned with 6-OHDA at 3 days after birth are behaviorally indistinguishable from control. Eating, drinking, grooming, and motor activity levels are equivalent to that of control rats – except that these rats are ~5% smaller. The latter alteration can be obviated by exchanging dams before the time of weaning (Brus et al., 1994).

### *DA-Agonist-induced behaviors of 6-OHDA-lesioned rats*

When challenged with the first or second dose (1 wk interval) of the DA $D_1$ agonist SKF 38393, locomotor and stereotyped behaviors were identical in control and lesioned rats. However, the third and subsequent dose of SKF 38393 produced a many-fold increase in locomotor and stereotyped activities in 6-OHDA-lesioned rats (Kostrzewa, 1995). The gradual induction of $D_1$ receptor supersensitivity is characterized as a *priming* phenomenon (Breese and Breese, 1998).

When challenged with the first or repeated doses (1 wk interval) of the DA $D_2$ agonist quinpirole, locomotor and stereotyped behaviors were identical in control and lesioned rats. However, the initial two doses of quinpirole produced heterotypic *priming* of $D_1$ receptors. Accordingly, if rats received two challenge treatments with quinpirole, the first dose of the $D_1$ agonist SKF 38393 produced a many-fold increase in locomotor and stereotyped activities (Breese and Breese, 1998).

### *Striatal in vivo microdialysate alterations in 6-OHDA-lesioned rats*

When challenged with the first dose of 3,4-L-dihydroxyphenylalanine (L-DOPA), there was a many-fold increase in locomotor and stereotyped activities in 6-OHDA-lesioned rats (Breese and Breese, 1998). When these rats were studied by *in vivo* microdialysis, L-DOPA acutely produced a marked increase in striatal extraneuronal (*i.e.*, microdialysate) levels of both DA and DOPAC in the 6-OHDA-lesioned rats. In fact, the overall L-DOPA-induced increase in striatal extraneuronal DA and DOPAC was much greater in lesioned vs control rats (Kostrzewa et al., 2005).

In an ongoing series of studies we have further found that acute L-DOPA treatment did NOT increase the levels of hydroxyl radical ($HO^{\bullet}$) in either striatal tissue or in striatal *in vivo* microdialysates (Kostrzewa et al., 2000). The implication of these findings is that L-DOPA is not likely to accelerate DA neuronal damage and therefore, L-DOPA

is not likely to accelerate the progression of PD.

### *Neurochemical and neuroanatomical effects on striatal 5-HT innervation*

In rats treated at 3 days after birth with 6-OHDA, there is 5-HT (serotonin) fiber hyperinnervation of striatum, observed histochemically as an increase in the number of tryptophan hydroxylase-immunopositive fibers. Accompanying this neuroanatomical change is a 50–100% increase in the striatal content of both 5-HT and its major metabolite 5-HIAA (5-hydroxyindoleacetic acid). These rats are behaviorally supersensitive to $5\text{-HT}_2$ agonists (Brus et al., 1994; Kostrzewa et al., 1998). It is possible to prevent 5-HT fiber proliferation by adding a small dose of the 5-HT neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT), to the 6-OHDA solution at 3 days post-birth.

## Discussion

Rats lesioned with high-dose 6-OHDA at 3 days after birth represent a good model of severe PD, because the neuroanatomical and neurochemical indices in this model are representative of dopaminergic alterations in a human with severe PD. The rat model can be easily utilized, because 6-OHDA does not produce lethality. Also, the approx. 99% DA-denervation of striatum is reliable ($\pm 1\%$) and consistent. Survivability of the rats is not compromised, because eating, drinking, grooming, and locomotor activities are virtually unaltered – rats in adulthood are indistinguishable from controls. Moreover, the *in vivo* microdialysate studies with L-DOPA confirm that the L-DOPA-induced extraneuronal levels of DA and DOPAC are virtually identical to effects observed in rats that are DA-lesioned in adulthood.

## Conclusion

Rats lesioned neontally with 6-OHDA represent a near-ideal model of severe PD because of the reproducibility of the extent of DA fiber denervation, and absence of variability in effect and survivability of animals.

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